

# BUREAU OF WATER

South Carolina Department of Health and Environmental Control

## 2021 South Carolina Cyanotoxin Distribution Project

Technical Report No. 005-2023



2021 South Carolina Cyanotoxin Distribution Project  
Technical Report No. 005-2023  
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## Executive Summary

Harmful algal blooms (HABs) are an increasing concern in the United States and are generally caused by excessive growth of cyanobacteria, or blue-green algae. Cyanobacteria blooms can degrade water quality through increased water column turbidity that reduces light availability for ecologically important vegetation. Die-offs of these blooms can reduce oxygen levels that can lead to fish kills. Some cyanobacteria species produce toxins (cyanotoxins) that are harmful to humans, livestock, and wildlife. In high enough concentrations, algal blooms can also cause nuisance taste and odor issues in drinking water and increase the cost of water treatment.

In 2018, the South Carolina Department of Health and Environmental Control (SCDHEC) initiated the HABs Monitoring Program to investigate the effects that cyanotoxins have on human health and the environment within the State. This report presents the cyanotoxin work completed in 2021. In 2021, SCDHEC aimed to:

- Continue collecting baseline data for cyanotoxin distribution in State reservoirs and estuaries,
- Monitor drinking water intakes with a history of HABs and/or taste and odor issues,
- Issue recreational advisories for waterbodies that exceed SCDHEC's state standards, and
- Identify potential correlative relationships between cyanotoxin concentrations and other physicochemical water quality parameters.

In 2021, samples were collected and analyzed for microcystins from 101 monthly-monitored sites across South Carolina reservoirs, estuaries, and influent streams. Microcystin samples were collected from May 1 to October 31. Five (5) of the 101 stations were sampled starting in April due to a special nutrient study on Lake Murray. The monthly-monitored sites were coordinated with routine sampling conducted by SCDHEC regional field staff, which allowed data comparison to other parameters collected contemporaneously. In addition to monthly monitoring of lake and estuarine sites, samples were collected from an additional four (4) lakes at six (6) drinking water intakes with past algal issues, including taste and odor complaints. Eight (8) waterbodies were sampled in response to the occurrence of possible HAB conditions (event-driven samples) from March through November.

Monthly-monitoring concentrations were less than 1 microgram per liter ( $\mu\text{g/L}$ ) for microcystins. Concentrations greater than the analytical detection level ( $\geq 0.100 \mu\text{g/L}$  for ADDA ELISA method or  $\geq 0.016 \mu\text{g/L}$  for SAES ELISA method) were observed in 79% of samples analyzed for microcystins. Toxin concentrations in all monthly-monitoring samples were less than SCDHEC's recreational standard of 8  $\mu\text{g/L}$  for microcystins.

Microcystins were also detected at all six (6) drinking water intakes. The drinking water intakes at Lake Murray (City of Columbia and City of West Columbia), Lake Rabon (Laurens Commission of Public Works (CPW)), and Lake Whelchel (Gaffney Board of Public Works (BPW)), had at least one (1) sample that exceeded USEPA 10-day drinking water health advisory value of 0.3  $\mu\text{g/L}$  for microcystins; however, the treatment processes at the drinking water facilities reduced microcystin concentrations to less than the detection limit.

Recreational advisories were issued in 2021 at Lake Whelchel, Lake Rabon, and Lake Wylie for toxin concentrations greater than SCDHEC's state recreational standard. Advisories were removed once the microcystin concentrations were below 8  $\mu\text{g/L}$  and the blooms had dissipated. Recreational watches were

also issued in 2021 at Goose Creek Reservoir and Lake Wylie. Recreational watches are issued when a potential toxin producing bloom is identified on a waterbody but microcystin or cylindrospermopsin concentrations are less than SCDHEC's state standards, or the identified algal species could potentially be producing algal toxins, such as anatoxin and saxitoxin, that are not in SCDHEC's state standards.

Correlation analyses were conducted for monthly-monitoring microcystin concentration data for Lake Greenwood, Lake Hartwell, Lake Murray, and Lake Wateree. No strong relationships were determined for microcystin concentrations and water quality parameters including dissolved oxygen, pH, temperature, total phosphorous, nitrogen: phosphorus ratio, and chlorophyll *a* for any of the lakes.

This assessment builds on the 2018, 2019, and 2020 studies and broadens the baseline understanding of cyanotoxin distributions across the State. In 2021, the HABs Monitoring Program launched a HAB Monitoring GIS App to allow the public to view monitoring stations, data, and current recreational watches and/or advisories. Future goals of the HABs Monitoring Program include evaluating additional toxins, such as anatoxin and saxitoxin, and expanding sampling to large rivers and streams. This will further enhance the State's growing understanding of cyanotoxin distributions.

## Introduction and Background

Harmful algal blooms (HABs) are an increasing concern in U.S. waters. These blooms occur when algae grow excessively in response to elevated nutrient concentrations, typically from non-point source runoff due to a variety of land-uses. In high enough densities, blue-green algae, or cyanobacteria, can impact aquatic life and human health by degrading water quality and producing cyanotoxins. There is growing recognition of the need for increased monitoring of cyanotoxin concentrations in waterbodies and in the water treatment process (Jetto, Grover, & Krantxberg, 2015). The U.S. Environmental Protection Agency (USEPA) has issued health advisory criteria (U.S. Environmental Protection Agency, 2019) and recreational advisory criteria (U.S. Environmental Protection Agency, 2015b,c) for two (2) cyanotoxins (microcystins and cylindrospermopsin). Exposure to high levels of microcystins can lead to liver, reproductive, developmental, kidney, and gastrointestinal effects (U.S. Environmental Protection Agency, 2019). Exposure to high levels of cylindrospermopsin can affect the liver, kidneys, and potential deformation of red blood cells (U.S. Environmental Protection Agency, 2019).

The South Carolina Department of Health and Environmental Control (SCDHEC) has maintained a robust surface water monitoring network since the 1950s. With the advancement of cyanotoxin analytical methods, SCDHEC established the HABs Monitoring Program in 2018 to monitor cyanotoxins statewide. A primary objective of the HABs Monitoring Program is to establish a baseline and context for interpretation of cyanotoxin concentrations in South Carolina’s waters, which was accomplished with the adoption of the USEPA’s recreational advisory criteria (Table 1) in SCDHEC’s State standards in 2020.

**Table 1:** SCDHEC recreational water quality advisory criteria for microcystins and cylindrospermopsin. Recreational water activities include swimming, rowing, fishing, boating, etc.

SCDHEC Recreational Water Quality Advisory Criteria		
Microcystin Concentration (µg/L) <sup>a, b</sup>	Cylindrospermopsin Concentration (µg/L) <sup>a, b</sup>	Duration
8	15	Recreational advisories will remain in place until two (2) consecutive samples report back as less than the advisory criteria

a. SCDHEC Regulation 61-68

b. µg/L = micrograms per liter (parts per billion)

## Purpose of Assessment

The purpose of the 2021 assessment was to examine cyanotoxin distributions in South Carolina reservoirs and estuaries to determine potential risks for recreational and aquatic life uses for waterbodies of the State. Cyanotoxin concentrations were also compared to USEPA drinking water health advisories (Table 2) to identify potential hazards to drinking water facilities. The data were used to identify reservoirs of potential concern and will guide future assessment activities. In 2021, monitoring activities primarily focused on analyzing microcystin toxins based on results from the previous three (3) years. Species having the potential to produce cylindrospermopsin were identified at two (2) lakes in 2021 as a result of sampling an algal bloom due to complaints.

**Table 2:** USEPA 10-day health advisory values for microcystins and cylindrospermopsin in drinking water.

Cyanotoxin	USEPA 10-day Drinking Water Health Advisory <sup>a, b</sup>	
	Bottle Fed Infants and pre-school children (µg/L)	School age children and adults (µg/L)
Microcystins	0.3	1.6
Cylindrospermopsin	0.7	3.0

a. U.S. Environmental Protection Agency, 2015b, c

b. µg/L = micrograms per liter (parts per billion)

## Methods

SCDHEC Bureau of Water (BOW) Aquatic Science Programs (ASP) collected cyanotoxin samples from March 2021 to December 2021 for microcystins. Three (3) types of sampling were conducted as part of the 2021 study: monthly-monitoring at waterbodies throughout the State, sampling at drinking water intakes with a history of algal issues (drinking water lake source monitoring), and sampling in response to complaints (event-driven). The event-driven sampling included visually observed algal blooms and a fish kill in response to citizen and stakeholder complaints. A total of 19 freshwater bodies and 39 estuaries and influent streams were sampled during the monthly-monitoring component, six (6) drinking water lake intakes, and complaint samples were collected at eight (8) different water bodies due to event-driven responses. An extensive HAB occurred on Lake Wylie, one of the water bodies that was sampled due to an event-driven response, and over 60 samples were collected over the duration of the bloom. In 2020, the USEPA criteria for recreational water quality and swimming advisories for microcystins and cylindrospermopsin were adopted as State water quality standards.

### Monthly-Monitoring

One hundred and one (101) sites were sampled monthly from May 2021 to October 2021 (Table 3 and Figure 1). These sites were selected from the 2021 list of Ambient Water Quality Monitoring Program sites (SCDHEC, 2021b). The 2021 Ambient Water Quality Monitoring Program collected monthly samples from a total of 243 Base Sites for water quality parameters including temperature, chlorophyll *a*, nutrients, metals, etc. providing an opportunity to compare cyanotoxin results to other water quality parameters. Five (5) of the 101 sites were sampled from April 2021 to October 2021 due to a special nutrient study being conducted on Lake Murray, which were sampled according to SCDHEC BOW technical report No. 003-2022 (SCDHEC, 2022b).

A total of 595 samples were analyzed for microcystins. Sample collection, field analysis, handling, preservation, and Chain of Custody (COC) followed SCDHEC Determination of Total Microcystins and Cylindrospermopsin in Ambient Water Standard Operating Procedure (SOP) (Appendix 1). The field manager oversaw the transportation of the samples and the COCs to the SCDHEC ASP laboratory. Samples were frozen at -20°C for a holding time not to exceed two (2) weeks.

Samples were analyzed for microcystins using the Enzyme Linked Immunosorbent Assay (ELISA) technique described in SCDHEC Determination of Total Microcystins and Cylindrospermopsin in Ambient Water SOP (Appendix 1). The analysis is based on USEPA method 546 (U.S. Environmental Protection Agency, 2015a)



with guidance from the assay provider, Abraxis. Microcytins/Nodularins ADDA ELISA and SAES ELISA plates were used for this analysis, with detection limits of 0.100 ug/L and 0.016 ug/L, respectively.

**Table 3:** Sampling site locations for monthly-monitoring.

Site	Regional Lab	Description	Latitude	Longitude
B-327	Midlands	Monticello Lake	34.3297	-81.3026
B-339	Greenville	Lake Bowen	35.1128	-82.0455
B-345	Midlands	Parr Reservoir	34.2621	-81.3354
CL-019	Greenville	Lake Jocassee	34.9599	-82.9236
CL-041	Greenville	J. Strom Thurmond	33.6699	-82.2076
CL-069	Midlands	Langley Pond	33.5223	-81.8432
CL-089	Midlands	Lake Wateree	34.3368	-80.7049
CSTL-102	Charleston	Ashley River	32.9584	-80.2010
CSTL-107	Beaufort	Coosawhatchie River	32.5883	-80.9238
CW-016F	Lancaster	Fishing Creek Reservoir	34.6777	-80.8772
CW-033	Midlands	Cedar Creek Reservoir	34.5426	-80.8777
CW-057	Lancaster	Fishing Creek Reservoir	34.6053	-80.8910
CW-174	Midlands	Cedar Creek Reservoir	34.5581	-80.8917
CW-197	Midlands	Lake Wylie	35.1376	-81.0594
CW-201	Midlands	Lake Wylie	35.0281	-81.0477
CW-207	Midlands	Lake Wateree	34.4025	-80.7884
CW-207B	Midlands	Lake Wateree	34.4039	-80.7827
CW-208	Midlands	Lake Wateree	34.4219	-80.8674
CW-230	Midlands	Lake Wylie	35.0225	-81.0087
CW-231	Midlands	Lake Wateree	34.5365	-80.8749
LCR-02	Midlands	Lake Wateree	34.4858	-80.8998
LCR-03	Midlands	Lake Wateree	34.4254	-80.8439
LCR-04	Midlands	Fishing Creek Reservoir	34.6204	-80.8862
MD-001	Beaufort	Beaufort River	32.4456	-80.6632
MD-004	Beaufort	Beaufort River	32.3653	-80.6779
MD-043	Charleston	Cooper River	32.9629	-79.9212
MD-045	Charleston	Cooper River	32.8453	-79.9335
MD-049	Charleston	Ashley River	32.8758	-80.0815
MD-052	Charleston	Ashley River	32.7966	-79.9719
MD-069	Charleston	Intracoastal Waterway	32.7728	-79.8422
MD-077	Florence	Sampit River	33.3574	-79.2940
MD-115	Charleston	Wando River	32.9228	-79.9273
MD-116	Beaufort	Broad River	32.3848	-80.7838
MD-117	Beaufort	Chechessee	32.3741	-80.8361
MD-118	Beaufort	New River	32.2360	-81.0129
MD-120	Beaufort	Dawho River	32.6366	-80.3418
MD-125	Florence	Intracoastal Waterway	33.8533	-78.6539
MD-129	Beaufort	Great Swamp	32.4061	-81.0187
MD-130	Charleston	Folly River	32.6596	-79.9433
MD-142	Florence	Waccamaw River	33.4083	-79.2171
MD-173	Beaufort	May River	32.2104	-80.8423

<b>Site</b>	<b>Regional Lab</b>	<b>Description</b>	<b>Latitude</b>	<b>Longitude</b>
MD-174	Beaufort	Broad Creek	32.1804	-80.7740
MD-176	Beaufort	Colleton River	32.3323	-80.8774
MD-202	Charleston	Stono River	32.7857	-80.1075
MD-206	Charleston	Stono River	32.6744	-80.0046
MD-209	Charleston	Bohicket Creek	32.6223	-80.1643
MD-248	Charleston	Cooper River	32.8905	-79.9627
MD-252	Beaufort	Combahee River	32.5643	-80.5570
MD-253	Beaufort	Ashepoo River	32.5330	-80.4484
MD-256	Beaufort	Unnamed Creek	32.3399	-80.5078
MD-257	Beaufort	Ramshorn Creek	32.1288	-80.8890
MD-258	Beaufort	Ramshorn Creek	32.1110	-80.8986
MD-259	Beaufort	Wright River	32.0943	-80.9489
MD-260	Beaufort	S. Edisto River	32.5673	-80.3901
MD-261	Charleston	Yonges Island Creek	32.6947	-80.2229
MD-262	Charleston	N. Edisto River	32.6059	-80.2293
MD-264	Charleston	Wando River	32.8584	-79.8959
MD-266	Charleston	Casino Creek	33.0751	-79.3941
MD-267	Charleston	Five Fathom Creek	33.0366	-79.4769
MD-269	Charleston	Sewee Bay	32.9367	-79.6550
MD-271	Charleston	Hamlin Sound	32.8269	-79.7746
MD-273	Charleston	Kiawah River	32.6080	-80.1274
MD-275	Florence	Pee Dee River	33.4222	-79.2246
MD-277	Florence	Parsonnage Creek	33.5529	-79.0339
MD-278	Florence	Winyah Bay	33.2735	-79.0340
MD-281	Beaufort	Parrot Creek	32.4953	-80.5553
MD-282	Beaufort	Morgan River	32.4438	-80.6069
PD-325	Florence	Black River	33.4138	-79.2504
PD-327	Lancaster	Lake Robinson	34.4675	-80.1698
RL-19154	Midlands	Lake Murray	34.0695	-81.6186
S-022	Greenville	Lake Greenwood	34.3278	-82.0849
S-024	Greenville	Lake Greenwood	34.3079	-82.1101
S-131	Greenville	Lake Greenwood	34.2791	-82.0587
S-211	Midlands	Lake Murray	34.0984	-81.4765
S-213	Midlands	Lake Murray	34.1251	-81.4337
S-222	Midlands	Lake Murray	34.0802	-81.5625
S-223	Midlands	Lake Murray	34.0997	-81.5683
S-279	Midlands	Lake Murray	34.0763	-81.4724
S-280	Midlands	Lake Murray	34.0713	-81.3942
S-308	Midlands	Lake Greenwood	34.3467	-82.1088
S-309	Midlands	Lake Murray	34.1315	-81.6048
S-310	Midlands	Lake Murray	34.1151	-81.5999
S-311	Greenville	Boyd Mill Pond	34.4547	-82.2019
S-326	Midlands	Lake Murray	34.0682	-81.5869
ST-005	Florence	Santee River	33.2091	-79.3839
ST-006	Charleston	Santee River	33.1839	-79.4058

Site	Regional Lab	Description	Latitude	Longitude
SV-098	Greenville	Lake Russell	34.0704	-82.6429
SV-200	Greenville	Lake Hartwell	34.6117	-83.2262
SV-236	Greenville	Lake Hartwell	34.5954	-82.9078
SV-268	Greenville	Lake Hartwell	34.5972	-82.8218
SV-331	Greenville	Lake Secession	34.3319	-82.5758
SV-335	Greenville	Lake Jocassee	35.0320	-82.9151
SV-336	Greenville	Lake Jocassee	34.9959	-82.9793
SV-338	Greenville	Lake Keowee	34.8269	-82.8977
SV-339	Greenville	Lake Hartwell	34.5112	-82.8098
SV-340	Greenville	Lake Hartwell	34.4032	-82.8391
SV-357	Greenville	Lake Russell	34.1920	-82.6309
SV-361	Greenville	Lake Keowee	34.7339	-82.9183
SV-363	Greenville	Lake Hartwell	34.4800	-82.9454
SV-372	Greenville	Stephens Creek Reservoir	33.5928	-82.1233
SV-374	Greenville	Lake Hartwell	34.5721	-82.8299

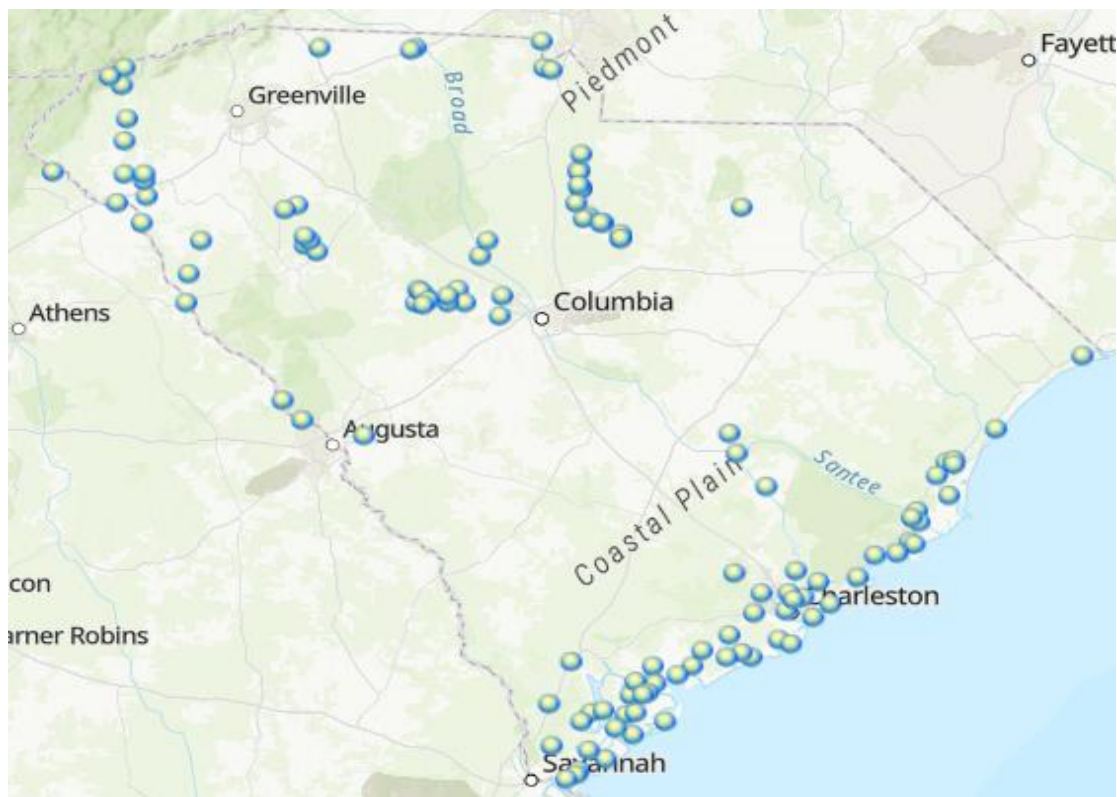


Figure 1: 2021 monthly-monitoring sampling site locations.

## Drinking Water Lake Source Monitoring

Four (4) lakes were sampled monthly from May through September 2021 in proximity to intakes for six (6) different drinking water facilities (Table 4). The lakes and drinking water intake sampling sites were selected based on previous algal issues and taste and odor complaints. A total of 24 samples were collected from the drinking water lakes and analyzed for microcystins. Sampling was focused near the drinking water facility intakes; however, additional samples were collected at other parts of the lakes if algal blooms were observed.

Drinking water sample collection, field analysis, handling, preservation, and laboratory analysis followed the same procedures as described above in the Monthly-Monitoring section.

**Table 4:** Sampling site locations for four (4) lakes that were monitored at their respective drinking water source intakes.

Lake	Drinking Water Facility	Latitude	Longitude
Lake Wateree	Lugoff Elgin Water Authority	34.3328	-80.7067
	City of Camden	34.3569	-80.7038
Lake Murray	City of Columbia	34.0215	-81.2326
	City of West Columbia	34.0978	-81.2313
Lake Rabon	Laurens Commissions of Public Works	34.4785	-82.1398
Lake Whelchel	Gaffney Board of Public Works	35.1079	-81.6222

## Event-Driven Samples

Eight (8) waterbodies were sampled in response to complaints reporting algal blooms, fish kills, and/or taste and odor issues during the 2021 sampling season. Sample locations are described in Table 6 below for seven (7) of the water bodies. Sampling on Lake Wylie occurred at 29 different locations due to the extensive HAB that occurred in 2021 (Figure 6). Toxin samples and/or phytoplankton tow nets were collected after a complaint was received. Samples were observed under the microscope for algal identification at the SCDHEC ASP laboratory and analyzed for microcystins and/or cylindrospermopsin if the species identified was a potential toxin producing species.

Sample collection, field analysis, handling, and preservation followed the same procedures as described above in the Monthly-Monitoring section. Samples identified with cyanobacteria were analyzed via the same procedures as described above in the Monthly-Monitoring section.

## Advisories and Watches

In 2021, recreational advisories were issued when one (1) or more sample exceeded SCDHEC's state standards for microcystins and/or cylindrospermopsin toxins (Table 1). If a recreational advisory was issued on a waterbody with a drinking water intake, drinking water providers were contacted and recommended to have the finished drinking water tested for toxins. Recreational advisories remained in place until a cyanotoxin result below the recreational state standard was measured and the bloom had dissipated. The public was notified about recreational advisories that were issued or lifted via press

releases and postings on the SCDHEC HABs webpage: <https://scdhec.gov/environment/your-water-coast/harmful-algal-blooms>.

Recreational watches were also issued when a potential toxin producing bloom was identified on a waterbody but toxins for microcystin or cylindrospermopsin were less than SCDHEC’s state standards, or the identified algal specie could potentially be producing algal toxins, such as anatoxin and saxitoxin, that are not in SCDHEC’s state standards. Recreational watches were monitored monthly and were removed once the bloom has dissipated.

Recreational advisories and watches were posted on the Harmful Algal Bloom Monitoring GIS Application: [https://gis.dhec.sc.gov/hab\\_viewer](https://gis.dhec.sc.gov/hab_viewer)

### Quality Assurance/ Quality Control

In total, 591 of the 595 samples analyzed for microcystins in 2021 passed quality control requirements. Quality Control Requirements can be found in section 10.5 of SCDHEC’s Determination of Total Microcystins and Cylindrospermopsin in Ambient Water SOP (Appendix 1). SCDHEC also participated in the Abraxis Cyanotoxins Proficiency Testing Program for recreational water as a check on the accuracy of ASP’s routine sample analysis. Performance was evaluated by calculating a z-score metric based on the analysis results of three (3) surface water standards fortified with purified Microcystin-LR, Microcystin-RR, Microcystin-YR, and/or nodularins (toxins produced by *Nodularia sp.*, a cyanobacterium). The z-score metric is as follows:

$$z = \frac{(x - X)}{\sigma}$$

Where:

- z= the z score (Standard score)
- x= the reported value of analyte
- X= the assigned value, the best estimate of the *true* concentration
- σ= the estimate of variation (proficiency standard deviation)

The following interpretations for z-scores in proficiency testing schemes are recommended:

Results Obtained	Rating
$z \leq 2$	Satisfactory
$2 < z < 3$	Questionable
$z \geq 3$	Unsatisfactory

The results for SCDHEC’s proficiency testing for each of the three (3) samples are listed in the table below.

Sample Number	Result (µg/L) <sup>a</sup>	Z-Score	Evaluation
1	0.716	0.29	Satisfactory
2	8.05	0.20	Satisfactory
3	9.23	1.79	Satisfactory

a. µg/L = micrograms per liter (parts per billion)

## Statistical Analyses

Pearson correlation coefficients were calculated to determine if there were linear relationships between concentrations of microcystins and pH, dissolved oxygen (mg/L), temperature (°C), total phosphorous (mg/L), N:P ratio, and chlorophyll *a* (µg/L) in water bodies that met the sample size requirement of three (3) detectable samples per month. Only detectable data (toxin concentration values greater than or equal to the method detection limit) were used for analyses. Microcystin concentration data were considered detectable when result(s) were  $\geq 0.016$  µg/L for SAES ELISA plates and  $\geq 0.100$  µg/L for ADDA ELISA plates.

Fifty-eight (58) bodies across the State were sampled as part of the 2021 monthly-monitoring program. Due to different hydrologic characteristics among the water bodies, lakes were analyzed individually. Water bodies meeting the minimum sample size requirement (three (3) detectable samples per month) over the course of six (6) months include Lake Greenwood, Lake Hartwell, Lake Murray, and Lake Wateree.

Pearson correlation matrix output values range from -1 to 1, where values closer to -1 indicate a strong inverse relationship and values closer to 1 indicate a strong positive relationship. Matrix values that are closer to zero indicates no linear relationship. All data analyses were made using Microsoft Excel.

## Results

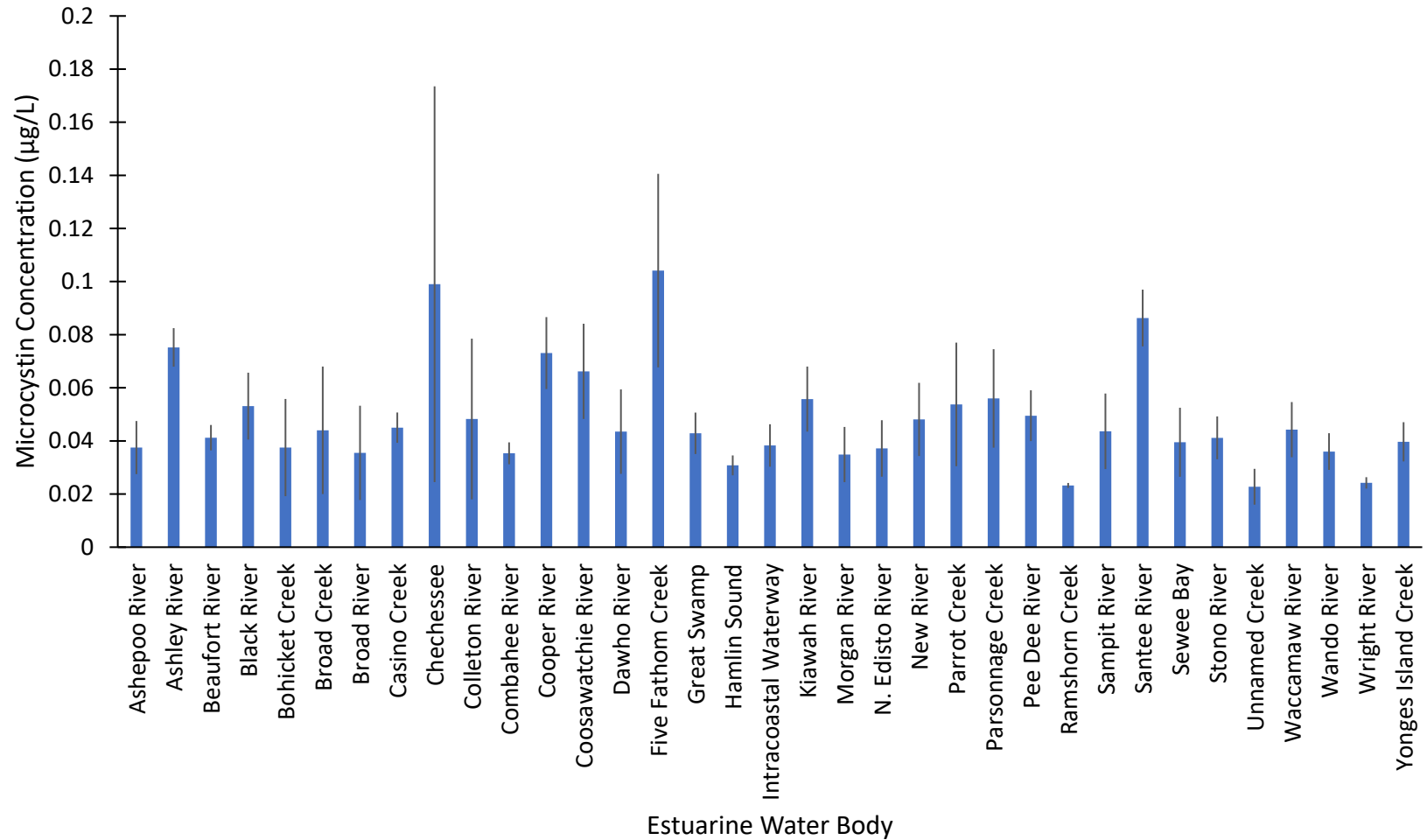
### Monthly-Monitoring

From April 2021 through October 2021, a total of 595 samples were collected for microcystins. Of the 591 samples meeting QA/QC guidelines for microcystins, 79% had concentrations greater than or equal to the method detection limit. The maximum microcystin concentration was 0.500 µg/L at station S-326 on Lake Murray in April 2021. All monthly-monitoring microcystin concentrations were less than 1 µg/L and all microcystin concentrations were less than the SCDHEC recreational action level of 8 µg/L.

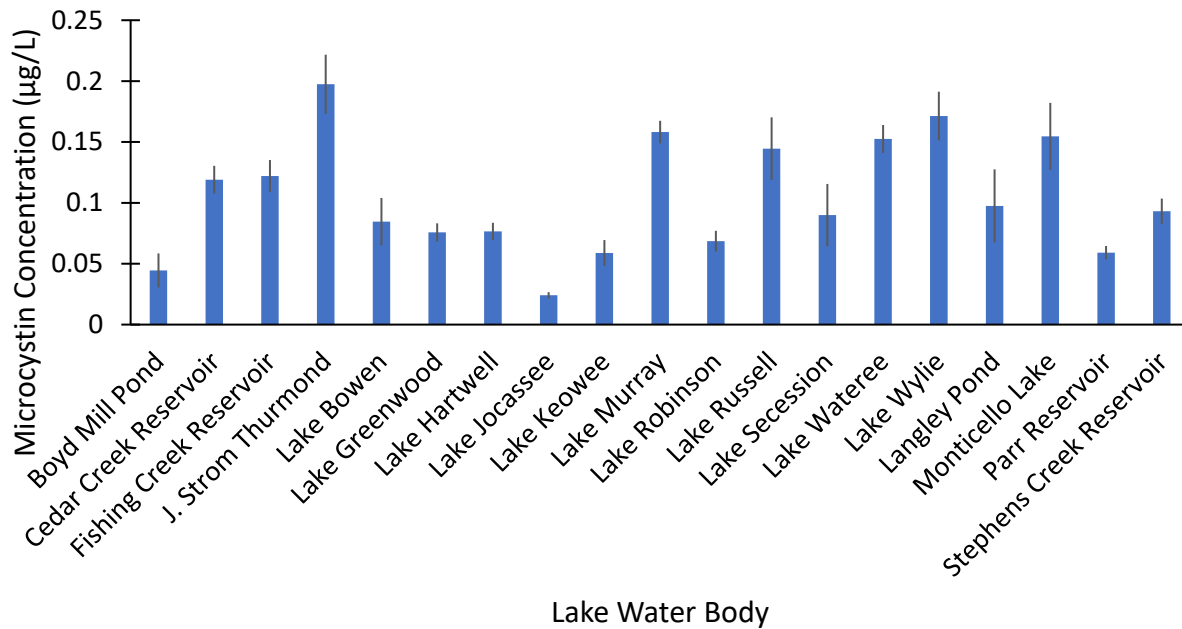
A total of 39 estuarine sites were sampled at 49 different sites during the 2021 monitoring season. Thirty-five (35) of the 39 estuarine sites had more than one (1) sample with detectable amounts of microcystins (Figure 2). Five Fathom Creek had the highest average microcystin concentration (mean ( $\bar{x}$ )=0.104 µg/L, standard error (SE)=0.036). Unnamed Creek, between Harbor River and Story River in Beaufort County, and Ramshorn Creek had the lowest average microcystin concentration ( $\bar{x}$ =0.023 µg/L, Unnamed Creek SE=0.008 and Ramshorn Creek SE= 0.001).

All 19 freshwater lakes had more than one (1) sample with detectable amounts of microcystins (Figure 3). J. Strom Thurmond Lake had the highest average microcystin concentration ( $\bar{x}$ =0.197 µg/L, SE=0.024); Lake Jocassee had the lowest average microcystin concentration ( $\bar{x}$ =0.024 µg/L, SE=0.003). Refer to Appendix 2 to see the microcystin concentrations of individual sites analyzed each month, organized based on lake location.

Microcystins did not strongly correlate with dissolved oxygen, pH, temperature, total phosphorous, N:P ratio, or chlorophyll *a* in Lake Greenwood, Lake Hartwell, Lake Murray, and Lake Wateree with coefficients ranging from -0.49 to 0.55 (Table 5).



**Figure 2:** Average detectable microcystin concentrations (µg/L) per estuarine site sampled in 2021. There were 35 estuarine sites that had more than one (1) sample with quantifiable concentrations. The error bars represent +/- one (1) standard error.



**Figure 3:** Average detectable microcystin concentrations (µg/L) per freshwater lake in 2021. There were 19 lakes that had more than one (1) sample with quantifiable concentrations. The error bars represent +/- one (1) standard error.

**Table 5:** Pearson correlation coefficient results comparing microcystin concentrations (µg/L) in Lake Greenwood, Lake Hartwell, Lake Murray, and Lake Wateree to dissolved oxygen (mg/L), pH, temperature (°C), total phosphorous (mg/L), N:P ratio, and chlorophyll *a* (µg/L).

Water Body	Microcystin Concentrations Correlation for Respective Water Quality Parameters					
	Dissolved Oxygen	pH	Temperature	Total Phosphorous	N:P	Chlorophyll <i>a</i>
Lake Greenwood	-0.49	-0.33	0.55	-0.14	-0.32	-0.01
Lake Hartwell	0.02	0.33	0.31	-0.34	-0.32	-0.06
Lake Murray	0.00	0.12	0.13	-0.17	0.16	-0.23
Lake Wateree	0.11	0.14	-0.10	-0.10	-0.15	0.51

**Summary of Monthly-Monitoring Findings**

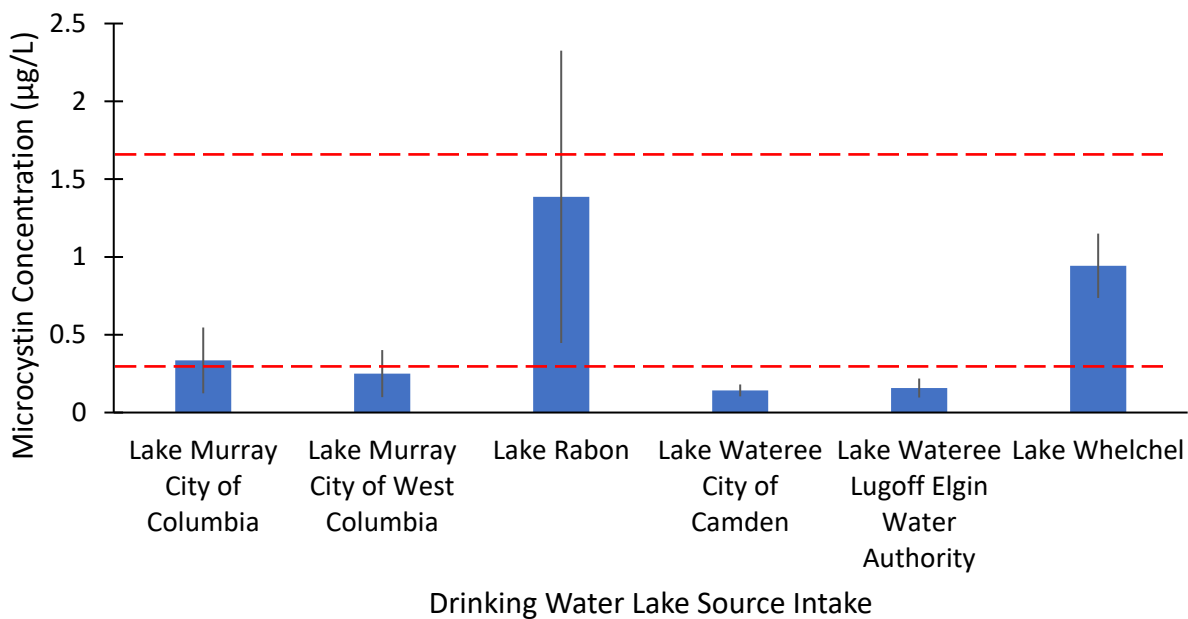
- 79% of the 591 samples analyzed for microcystins were detectable ( $\geq 0.100$  µg/L for ADDA ELISA or  $\geq 0.016$  µg/L for SAES ELISA method).



- All microcystin samples were less than the SCDHEC recommended recreational action level of 8 µg/L.
- There were no strong correlations between microcystin concentrations and dissolved oxygen, pH, temperature, total phosphorous, N:P ratio, and chlorophyll *a* in Lake Greenwood, Lake Hartwell, Lake Murray, or Lake Wateree.

### Drinking Water Lake Source Monitoring

From May through December 2021, 24 samples were collected for microcystins at four (4) different lakes for six (6) different drinking water facilities. Samples collected near the Laurens CPW drinking water intake at Lake Rabon had the highest average microcystin concentration ( $\bar{x}$ =1.39 µg/L, SE=2.49); the City of Camden drinking water intake samples at Lake Wateree had the lowest average microcystin concentration ( $\bar{x}$ =0.142 µg/L, SE=0.054). Lake Wateree (City of Camden and Lugoff-Elgin) samples were below the USEPA 10-day drinking water health advisory values of 0.3 µg/L for bottle fed infants and pre-school aged children and 1.6 µg/L for school age children and adults (Figure 4). Both intakes at Lake Murray (City of Columbia and City of West Columbia) had one (1) sample with a microcystin concentration above 0.3 µg/L. Six (6) samples taken near the Lake Rabon (Laurens CPW) drinking water intake had a microcystin concentration above 0.3 µg/L and one (1) sample was above 1.6 µg/L (microcystin concentration was 7.01 µg/L). All samples collected near the Lake Whelchel (Gaffney BPW) drinking water intake had a microcystin concentration above 0.3 µg/L, and one (1) sample was above 1.6 µg/L (microcystin concentration was 2.49 µg/L). The treatment processes at all drinking water intakes were able to remove the toxins and they were not detected in the finished drinking water.



**Figure 4:** Average detectable microcystin concentrations (µg/L) per drinking water source intake in 2021. There were four (4) lakes sampled for six (6) different drinking water facilities. The red lines indicate the USEPA drinking water 10-day health advisory values of 0.3 for bottle fed infants and pre-school children and 1.6 µg/L for school age children and adults. The error bars represent +/- one (1) standard error.

Sixty-one (61) additional drinking water lake samples were collected at algal blooms that occurred on Lake Rabon and Lake Whelchel. Of the sixty-one (61) additional samples collected, three (3) exceeded the recreational advisory of 8 µg/L at Lake Rabon and seven (7) exceeded the recreational advisory of 8 µg/L at Lake Whelchel. SCDHEC issued a recreational advisory for both lakes until the blooms dissipated. See the Advisories and Watches section below for more information on the additional drinking water lake samples.

#### Summary of Drinking Water Lake Source Sample Findings

- Microcystins were detected in samples collected near all six (6) drinking water intakes in 2021 ( $\geq 0.100$  µg/L for ADDA ELISA or  $\geq 0.016$  µg/L for SAES ELISA method).
- Lake Wateree (City of Camden and Lugoff-Elgin) samples were below the USEPA 10-day drinking water health advisory values of 0.3 µg/L for bottle fed infants and pre-school aged children and 1.6 µg/L for school age children and adults.
- One (1) sample at both Lake Murray intakes (City of Columbia and City of West Columbia) had a microcystin concentration above 0.3 µg/L.
- Six (6) samples taken at the Lake Rabon drinking water intake had a microcystin concentration above 0.3 µg/L, and one (1) sample was above 1.6 µg/L (microcystin concentration was 7.01 µg/L).
- All samples collected at the Lake Whelchel (Gaffney BPW) drinking water intake had microcystin concentrations above 0.3 µg/L, with one (1) sample above 1.6 µg/L (microcystin concentration was 2.49 µg/L).
- The treatment process at all drinking water intakes were able to remove microcystins, and the toxin was not detected in the finished drinking water.

#### Event-Driven Samples

Throughout the 2021 season, the SCDHEC BOW ASP section received complaints on eight (8) waterbodies. One (1) of these waterbodies, Lake Wylie, had an extensive bloom with multiple complaints. See the Advisories and Watches section below for more information. The other seven (7) waterbodies had nine (9) potential HABs; five (5) of the HABs were identified as cyanobacteria blooms with the potential to produce microcystins. All five (5) cyanobacteria samples had detectable levels of microcystins (Table 6). The highest concentration of microcystins (0.474 µg/L) was at Cane Island in Beaufort, SC.

Two (2) of nine (9) complaint blooms had the potential to produce cylindrospermopsin toxins based on the types of species present in the samples. However, both samples had cylindrospermopsin levels less than the detection limit (0.040 µg/L).

**Table 6:** Description and microcystin concentration ( $\mu\text{g/L}$ ) results from 2021 algal bloom complaints with the associated date of the HAB. Microscopic images of cyanobacteria for four (4) of the designated blooms can be found in Appendix 3.

Sample Location	Sample Description	Collection Date	Microcystins ( $\mu\text{g/L}$ ) <sup>a</sup>	Cylindrospermopsin ( $\mu\text{g/L}$ ) <sup>a</sup>
Lake Murray (34. 078105, -81.371573)	<i>Cymbella sp.</i>	03/16/2021	N/A <sup>b</sup>	N/A <sup>b</sup>
Goose Creek Reservoir	<i>Aphanizomenon sp.</i> bloom <sup>c</sup>	04/08/2021	N/A <sup>b</sup>	BDL <sup>d</sup>
Camp Canaan	<i>Euglena sanguinea</i> <sup>c</sup>	05/13/2021	N/A <sup>b</sup>	N/A <sup>b</sup>
Lake Murray near site RL-19154	<i>Scytonema sp.</i> and/or <i>Lyngbya sp.</i>	05/18/2021	0.365	N/A <sup>b</sup>
Cane Island- Beaufort SC	Fish Kill- most likely due to low dissolved oxygen. <i>Oxyrrhis marina</i> bloom.	06/08/2021	0.474	N/A <sup>b</sup>
Marsh land off Lake Keowee	Organic material with <i>Spirogyra</i> and <i>Oscillatoria</i> present	06/22/2021	0.320	N/A <sup>b</sup>
Lake Wateree	<i>Phormidium</i> bloom <sup>c</sup>	07/09/2021	0.115	N/A <sup>b</sup>
Anne Springs Greenway	<i>Planktothrix sp.</i> And <i>Microcystis sp.</i> bloom	07/14/2021	0.100	N/A <sup>b</sup>
Goose Creek Reservoir	<i>Dolichospermum sp.</i> bloom <sup>c</sup>	11/17/2021	0.294	BDL <sup>d</sup>

- a.  $\mu\text{g/L}$  = micrograms per liter (parts per billion)
- b. N/A= Not Applicable
- c. Microscope image of the associated cyanobacteria can be found in Appendix 3
- d. BDL= below detection limit

#### Summary of Event-Driven Sample Findings

- Five (5) of the nine (9) HAB complaint samples were analyzed for microcystins, and all five (5) samples had detectable levels of microcystins ( $\geq 0.100 \mu\text{g/L}$  for ADDA ELISA or  $\geq 0.016 \mu\text{g/L}$  for SAES ELISA method).
- Two (2) of the nine (9) HAB complaint samples were analyzed for cylindrospermopsin toxins. Both samples were below the detection limit ( $\geq 0.040 \mu\text{g/L}$ ).

#### Advisories and Watches

The recommended USEPA recreational water quality and swimming advisory criteria for microcystins and cylindrospermopsin (Table 1) were adopted as enforceable State water quality standards in 2020. Recreational advisories were issued in 2021 at Lake Rabon, Lake Whelchel, and Lake Wylie for microcystin concentrations higher than SCDHEC's state standard of  $8 \mu\text{g/L}$ . The advisories were lifted once microcystin concentrations were below  $8 \mu\text{g/L}$  and the algal bloom had dissipated. See individual lake sections below for information on the advisories.

Recreational watches were issued for the first time in 2021 as a result of algal blooms on Goose Creek Reservoir and Lake Wylie (Appendix 4). Two (2) different recreational watches occurred on Goose Creek Reservoir beginning in April and November and multiple coves of Lake Wylie were under a recreational watch in October due to the presence of HABs. The watches on Goose Creek Reservoir and Lake Wylie did not result in recreational advisories.

#### Lake Rabon

An advisory was issued for Lake Rabon on July 16, 2021, following a sample with a microcystin concentration greater than 15 µg/L at a cove across the lake from the drinking water intake (34.481852, -82.141791). The advisory was lifted on September 7, 2021 when two (2) consecutive samples had microcystin concentrations below 8 µg/L. The last sample had a microcystin concentration of 0.50 µg/L.

#### Lake Whelchel

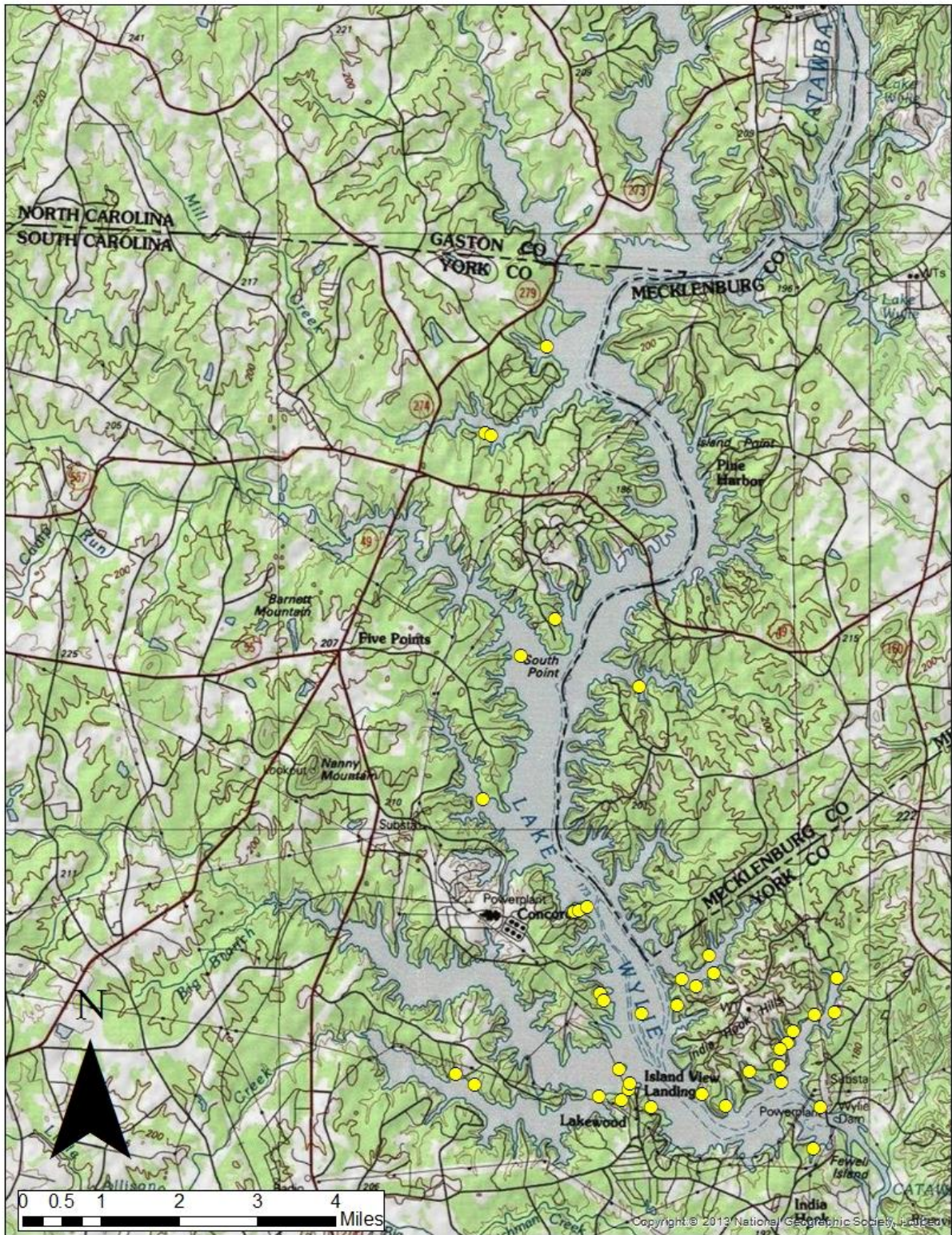
An advisory was issued for Lake Whelchel on July 22, 2021, following a sample with a microcystin concentration of greater than 20 µg/L near the Broad River outfall. The advisory was lifted on October 8, 2021 when two (2) consecutive samples had microcystin concentrations below 8 µg/L. The last sample had a microcystin concentration of 0.40 µg/L.

#### Lake Wylie

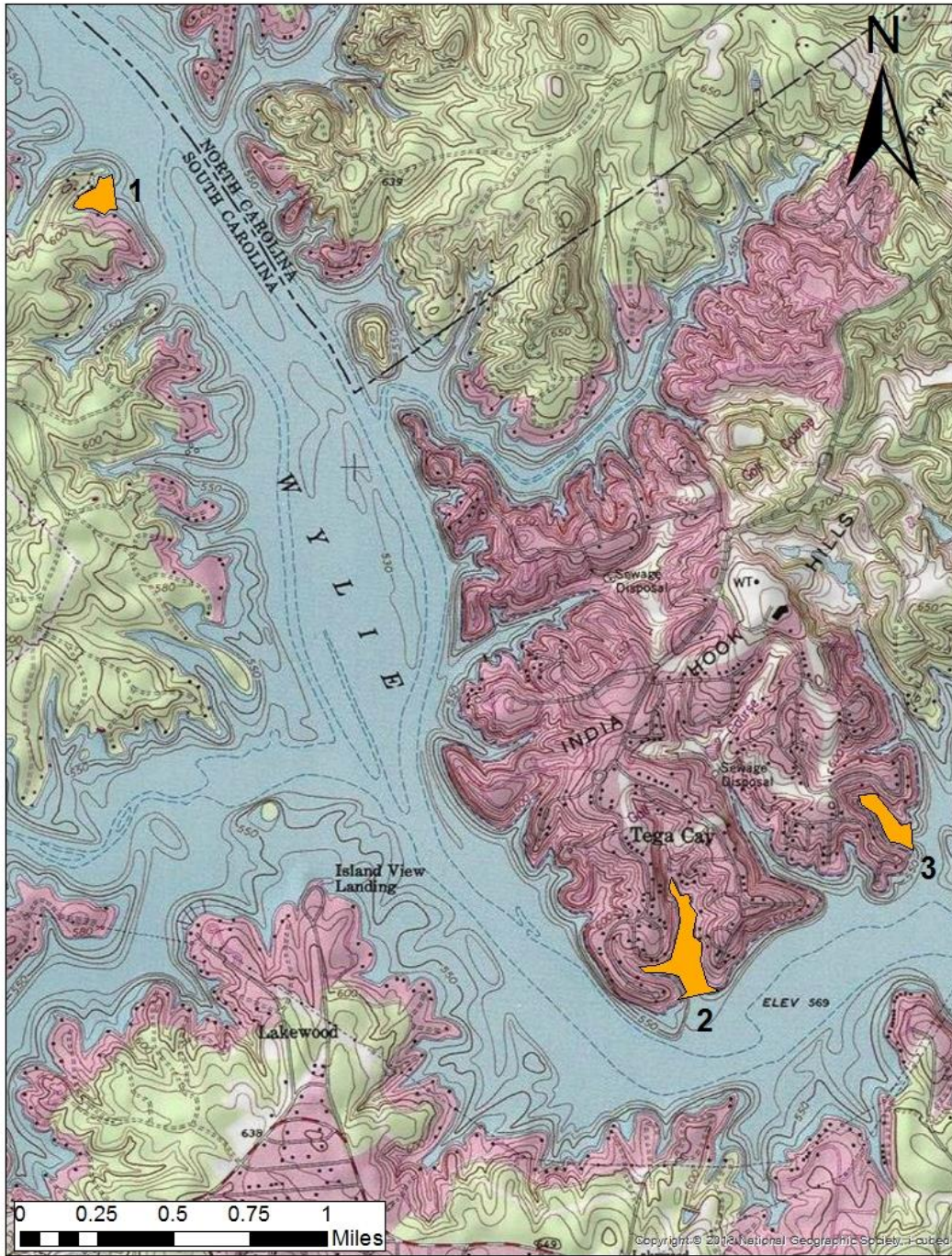
Lake Wylie lies on the border of South Carolina and North Carolina near Rock Hill, SC. Lake Wylie is a relatively large lake at 12,177 acres in total size, with 60% of the lake residing in South Carolina (Figure 5). Sampling on Lake Wylie began in August following a citizen complaint about a potential HAB on the lake. Recreational watches and advisories were issued for specific areas of Lake Wylie rather than the entire lake due to the large size of the lake and algal blooms localized to specific areas such as coves. See Appendix 5 for images from the Lake Wylie HAB.

Three (3) advisories were issued for Lake Wylie at three (3) different coves in the fall of 2021 (Figure 6, Table 7). The first advisory was issued at a cove between Clematis Trail and Acacia Road in late September following a sample with a microcystin concentration of greater than 8 µg/L. The other two (2) advisories were issued in early October following samples with microcystin concentrations greater than 8 µg/L. The highest microcystin concentration observed was 17.9 µg/L at the cove between Palmyra Drive and Molokai Drive. All three (3) advisories were lifted by mid-November when at least two (2) consecutive sampling events came back with samples less than 8 µg/L and the HAB was no longer present. Over the course of the Lake Wylie response, SCDHEC received 43 complaints from citizens pertaining to the HAB and over 60 samples were collected from approximately 39 different locations on the lake (Figure 5).

As a response to the HAB on Lake Wylie, a Lake Wylie HABs stakeholder group was created at the end of 2021 to discuss the algal bloom, each agency's response, and ways to ensure a more comprehensive response to future blooms between South Carolina and North Carolina. Participants of the Lake Wylie HABs stakeholder group included SCDHEC, Mecklenburg County (NC), North Carolina Department of Natural Resources (DNR), York County (SC), City of Tega Cay (SC), Catawba River Keeper (SC and NC), North Carolina Department of Environmental Quality, and Duke Energy (SC and NC).



**Figure 5:** Lake Wylie, a man-made lake, lies between South Carolina and North Carolina. Sampling locations during the 2021 Lake Wylie HAB are represented by yellow circles.



**Figure 6:** SCDHEC issued three (3) recreational advisories for three (3) covers, highlighted in orange, on Lake Wylie in 2021. The numbers next the advisories correspond to the respective advisories listed in Table 7.

**Table 7:** Three (3) recreational HAB advisories issued on Lake Wylie in 2021 due to microcystin concentrations  $\mu\text{g/L}$  greater than SCDHEC’s state standard of 8  $\mu\text{g/L}$ . Samples were routinely collected at the water body until the advisory was lifted. The initial and ending microcystin concentrations were when the advisory was issued and lifted, respectively.

Advisory Number	Lake Wylie Advisory Location	Advisory Issued	Advisory Lifted	Initial Microcystin Concentrations ( $\mu\text{g/L}$ ) <sup>a</sup>	Ending Microcystin Concentrations ( $\mu\text{g/L}$ ) <sup>a</sup>
1	Cove between Clematis Trail and Acacia Road	09/30/2021	11/16/2021	>10.0	0.00
2	Cove between Point Clear Drive and Marquesas Avenue	10/08/2021	11/16/2021	11.5	0.12
3	Cove between Palmyra Drive and Molokai Drive	10/08/2021	11/16/2021	17.9	0.12

a.  $\mu\text{g/L}$  = micrograms per liter (parts per billion)

#### Summary of Advisories and Watches

- Recreational advisories were issued in 2021 at Lake Rabon, Lake Whelchel, and Lake Wylie for microcystin concentrations higher than SCDHEC’s state standard of 8  $\mu\text{g/L}$ .
- Recreational watches were issued for the first time in 2021 as a result of algal blooms on Goose Creek Reservoir and Lake Wylie.

## Discussion and Conclusions

A primary goal of the HAB Monitoring Program is to establish cyanotoxin spatial distribution data in South Carolina waterbodies. These 2021 results have (a) contributed to a cyanotoxin concentration baseline for South Carolina waterbodies and (b) provided insight towards cyanotoxin presence/absence expectations. The total number of samples for microcystins increased by 14% from 2020 to 2021 and microcystins were detected in 79% of the samples that passed QA/QC. SCDHEC expanded the HABs Monitoring Program in 2021 by creating a Harmful Algal Bloom (HAB) Monitoring Application. The HAB Monitoring GIS App allows citizens to view sampling locations, data results, and any recreational advisories and/or watches that were occurring in the state.

Overall, the results from the 2021 monthly-monitoring for microcystins in lakes showed toxin concentrations less than 1  $\mu\text{g/L}$ , well below SCDHEC’s recreational standards of 8  $\mu\text{g/L}$ . Estuaries were monitored for cyanotoxins for the second consecutive year in 2021. While all microcystin concentrations for estuaries were below 1  $\mu\text{g/L}$ , these data are important milestones in establishing baseline toxin levels along the coast. The low cyanotoxin concentrations observed as part of the monthly-monitoring data suggest that generally recreational activities in South Carolina are not an immediate concern. Maintaining and expanding monthly-monitoring in the future field seasons will help in identifying localized elevated

cyanotoxin concentrations in various environments. A limitation of the monthly-monitoring sampling sites is that they are fixed open-water locations. Cyanobacteria blooms often occur in shallow coves or along shorelines.

The event-driven sampling is a more targeted component of the HAB Program, which provides insight into potential cyanotoxin producing HABs in nearshore environments. Microcystin concentrations in event-driven samples ranged from below detection limit to 0.474 µg/L. Two (2) complaint samples were tested for cylindrospermopsin but did not have any detectable limits of toxins. Recreational advisories were issued in 2021 for Lake Rabon, Lake Whelchel, and Lake Wylie. The longest advisory occurred at Lake Whelchel which lasted for approximately two (2) months. Lake Wylie was the largest lake, spanning between two (2) states and multiple counties, that SCDHEC has issued an advisory on since the formation of the HAB Monitoring Program in 2018. The complexity of Lake Wylie's location served as a catalyst in the formation of the Lake Wylie HABs stakeholder group in order to increase communication between SC and NC entities surrounding the lake.

SCDHEC's HAB Monitoring Program collaborated with six (6) drinking water facilities in 2021 to monitor drinking water intakes at four (4) lakes: Lake Murray, Lake Rabon, Lake Wateree and Lake Whelchel. Microcystins were detected at all drinking water intakes, but Lake Rabon, Lake Murray, and Lake Whelchel had the only drinking water intakes with at least one (1) sample greater than the USEPA 10-day drinking water health advisory value of 0.3 µg/L for bottle fed infants and pre-school aged children. The treatment process at Laurens CPW (Lake Rabon), Gaffney BPW (Lake Whelchel), and City of Columbia and West Columbia (Lake Murray) were able to remove the microcystins, and the toxin was not present in the finished drinking water. As HABs continue to expand and increase in frequency and duration, monitoring drinking water intakes and collaborating with drinking water facilities will continue to be a vital component of the HAB Monitoring Program.

No strong relationships were observed in the monthly-monitoring correlation results comparing microcystin concentrations to dissolved oxygen, pH, temperature, total phosphorus, N:P ratio, and chlorophyll *a* for Lake Greenwood, Lake Hartwell, Lake Murray, and Lake Wateree. The lack of a clear relationship among these monitoring variables is consistent with the analyses from the past three (3) seasons (SCDHEC, 2020a; SCDHEC, 2021a; SCDHEC, 2022a). The lack of a relationship suggests that the periodic occurrence of toxin producing cyanobacteria species is more complex than a single variable correlation in the same time and space (Davis, Berry, Boyer, & Gobler, 2009; Paerl & Otten, 2012; Wiltsie, Schnetzer, Green, Vander Borgh, & Fensin, 2018) or is related to environmental variables not routinely measured as part of the ambient monitoring program. Further, these lake-by-lake datasets are small and likely not robust enough for meaningful correlation. More data over the next several years will build on the past four (4) years of data and may provide a clearer understanding of patterns in cyanotoxin production.

In conclusion, the monthly-monitoring cyanotoxin results were generally lower than the SCDHEC state recreational standards, suggesting recreational activities in South Carolina were not an immediate concern. The 2021 season was the second full season for cyanotoxin monthly-monitoring in estuaries. While initial microcystin concentrations were low, continuing to monitor the estuarine environment in future years will improve and expand SCDHEC's understanding of harmful cyanobacteria presence along the coast. Other than the complaint sampling at Lake Wylie, there were no event driven samples where the microcystin or cylindrospermopsin concentrations exceeded SCDHEC recreational state standards.



SCDHEC issued recreational watches when a potential toxin producing bloom was identified on a waterbody but the toxin concentrations were less than SCDHEC's state standards or the algal species could potentially be producing algal toxins that are not in SCDHEC's state standards, such as anatoxin and saxitoxin. SCDHEC continued to work with drinking water facilities to monitor six (6) different drinking water intakes at four (4) lakes for microcystins. Microcystins were present at each drinking water intake, but the drinking water treatment successfully removed the toxin. Even though no strong correlations between microcystin concentrations and other environmental parameters were discerned in this assessment, a larger dataset over several years may provide better insight into relationships among these variables. The HAB Monitoring Program continues to work on educating South Carolina residents on HABs by creating the HAB Monitoring GIS Application. Future goals of the HABs Monitoring Program include expanding the statewide cyanotoxin study to include other toxins, such as anatoxin and saxitoxin as well as incorporating large rivers and streams into the cyanotoxin monitoring program.

#### Overall Summary:

- 2021 completed the fourth year of the HAB Monitoring Program. The data gathered in 2018, 2019, and 2020 will be used to inform future sampling plans and provide insights into lakes that the agency may consider monitoring more frequently.
- The monthly-monitoring sampling suggest no immediate concern for recreation activities due to the low concentrations of microcystins in open water settings.
- 2021 was the second full year for monthly-monitoring for microcystins of estuarine water bodies
- Recreational advisories were issued for Lake Rabon, Lake Whelchel, and Lake Wylie. Recreational watches were also issued when a potential toxin producing bloom was identified on a waterbody but was producing toxins for microcystin or cylindrospermopsin less than SCDHEC's state standards, or the identified algal specie could potentially be producing algal toxins, such as anatoxin and saxitoxin, that are not in SCDHEC's state standards.
- There were no strong correlations between microcystin concentrations and other parameters measured in Lake Greenwood, Lake Hartwell, Lake Murray, and Lake Wateree. Future analyses would benefit from a larger data set that also includes samples from algal blooms and examines a combination of factors.

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**Appendix 1: Standard Operating Procedure for Determination of Total Microcystins and  
Cylindrospermopsin in Ambient Water**




# Determination of Total Microcystins and Cylindrospermopsin in Ambient Water

## Bureau of Water- Aquatic Science Programs

**Revision 2.0**

**February 28, 2022**

  
\_\_\_\_\_  
Emily Bores  
Program Manager

02/28/22  
Date

  
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2022-03-01  
Date

  
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Jennifer Hughes Bureau Chief- BOW

3/1/2022  
Date

## **1. SCOPE AND APPLICATION**

### **1.1 Method Description**

These methods are used for the determination of algal toxins in ambient water, including (extracellular and intracellular) microcystins and cylindrospermopsin via enzyme-linked immunosorbent assay (ELISA). The detection limit for the Microcystin ADDA assay is 0.10 ppb ( $\mu\text{g/L}$ ) and the detection limit for the Microcystins ADDA SAES assay is 0.016 ppb ( $\mu\text{g/L}$ ). The detection limit for the Cylindrospermopsin assay is 0.040 ppb ( $\mu\text{g/L}$ ). The detection limit for using the seawater sample treatment solution for Cylindrospermopsin is 0.015ppb ( $\mu\text{g/L}$ ).

## **2. METHOD SUMMARY**

The method is an immunoassay for the quantitative and sensitive congener-independent detection of Microcystins and Nodularins and Cylindrospermopsin in ambient water samples. The testing is completed in a 96-well microtiter plate.

### **2.1 Microcystins**

The test is an indirect competitive ELISA for the congener-independent detection of Microcystins and Nodularins. It is based on the recognition of Microcystins, Nodularins, and their congeners by specific antibodies. Microcystins, nodularins, and their congeners when present in a sample and a Microcystins-protein analogue immobilized on the plate compete for binding sites of antibodies in solution. The plate is then washed and a second antibody-HRP label is added. After a second washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Microcystins present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

### **2.2 Cylindrospermopsin**

The test is a direct competitive ELISA for the detection of Cylindrospermopsin. It is based on the recognition of Cylindrospermopsin by specific antibodies. Cylindrospermopsin, when present in a sample, and a Cylindrospermopsin-HRP analogue compete for the binding sites of rabbit anti-Cylindrospermopsin antibodies in solution. The anti-Cylindrospermopsin antibodies are then bound by a second antibody (goat anti-rabbit) immobilized on the wells of the microtiter plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Cylindrospermopsin present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

### **3. DEFINITIONS**

#### **3.1 Analysis Batch**

Standards, samples, and quality control elements are assayed on a single 96-well plate using identical lots of reagents and wells. Each plate by definition is an Analysis Batch, regardless of the number of wells included. Quality control samples must be analyzed in each Analysis Batch at the frequencies prescribed. Each Analysis Batch includes the following elements:

- Calibration Standards
- Quality Controls
- Field samples (ambient water)

#### **3.2 Well Replicates**

Within the Analysis Batch, this method requires each calibration standard, field sample, and QC sample to be assayed in two wells. These two wells are called well replicates. Two values are associated with each well replicate: an absorbance measured by the plate reader, and a concentration calculated from this absorbance.

#### **3.3 Use of Well Replicate Absorbance Values**

For each set of well replicates, the percent coefficient of variation (%CV) is calculated from the two absorbance values. The %CV of the absorbance values for calibration standards must meet QC criteria. The %CV of the absorbance values for all field and QC samples must meet the limits. Refer to Table 2 for QC criteria.

#### **3.4 Use of Well Replicate Concentrations**

For each set of well replicates, the mean is calculated from the two concentration values. The mean concentration must be used for reporting field sample results. The mean must be used in all method calculation and for evaluating results against QC limits.

#### **3.5 Calibration Standards**

Solutions of Microcystin and Cylindrospermopsin toxins provided in the ELISA kit or prepared in the laboratory that are appropriate for the measurement range of the ELISA kit.

#### **3.6 Calibration Curve**

The calibration points are modelled using a four-parameter logistic function, relating concentration (x-axis) to the measured absorbance in the wells (y-axis). Note the inverse relationship between concentration and response. The zero calibration standard gives the highest absorbance and the highest calibration standard gives the lowest absorbance. Note also that the slope, or sensitivity, of the ELISA response is greatest in the middle of the curve and tends toward zero slope at extreme low and high concentrations.

### 3.7 Four-parameter Logistic Equation

$$y = \frac{(a - d)}{1 + \left(\frac{x}{c}\right)^b} + d$$

y= absorbance

x= concentration

a= absorbance at the bottom plateau

b= slope related term at the inflection point

c= concentration at the inflection point= EC<sub>50</sub>

d= absorbance at the top plateau

The coefficients, a, b, c, and d, are calculated by the data reduction software using regression analysis.

### 3.8 Quality Control Sample (QCS)

A solution containing microcystin toxins or cylindrospermopsin toxins at a known concentration that is obtained from a source different from the source of calibration standards. The purpose of the QCS is to verify the accuracy of the primary calibrations standards.

## 4. HEALTH AND SAFETY WARNINGS

### 4.1 Microcystins

The standard solution in the test kit contains small amounts of Microcystins. The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

### 4.2 Cylindrospermopsin

The standard solutions in the test kit contain small amounts of Cylindrospermopsin. The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

### 4.3 Cylindrospermopsin Seawater Sample Reagent

Irritant to skin and mucous membranes. May cause eye irritation in susceptible persons. The chemical, physical, and toxicological properties of this reagent have not been thoroughly investigated.

**4.4** Each laboratory is responsible for maintaining an awareness of OSHA regulations regarding safe handling of any chemicals used in this method. A reference file of Safety Data Sheets should be made available to all personnel involved in the analysis. Handle samples and standards using appropriate personal protective equipment.

## **5. INTERFERENCES**

- 5.1** Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test or QuikLyse. However, due to high variability of compounds that may be found in water samples, test interferences caused by matrix effects cannot be completely excluded.
- 5.2** Samples containing methanol must be diluted to a concentration <1% methanol to avoid matrix effects.
- 5.3** Mistakes in handling the test can cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, and extreme temperatures during the test performance (lower than 10°C or higher than 30°C). The assay procedure should be performed away from direct sunlight.
- 5.4** To avoid cross contamination between samples, do not reuse plastic syringes for filtering. Thoroughly clean glass containers if they are reused. Do not reuse septa from bottle containing ambient water samples.
- 5.5** As with any analytical technique, positive results requiring regulatory action should be confirmed by an alternative method.

## **6. SAMPLE HANDLING, PRESERVATION, AND STORAGE**

- 6.1** Collect samples in 500 mL polyethylene terephthalate glycol (PETG) containers with Polytetrafluoroethylene (PTFE) lined septa lids. Use of other types of plastic collection and/or storage containers may result in adsorptive loss of Microcystins, producing inaccurate (falsely low) results. Ambient water samples do not need to be treated after collection. Freeze samples upon arrival at the laboratory. Samples can be stored in the freezer for up to 2 weeks. When freezing, allow adequate volume for expansion and place the sample container on its side to prevent breakage.
- 6.2** Place samples on ice immediately. The temperature blank in the cooler must not exceed 10°C during the first 48 hours after collection. A temperature of greater than 10°C is acceptable if transit time is short and the samples do not have sufficient time to chill. In this case, examine the ice packs in the cooler. If they remain frozen, the samples are valid. Based on holding time (see section 6.1), refrigerate or freeze samples upon arrival to the laboratory.
- 6.3** Samples may be filter and assayed any time after lysing if within 14 days of collection. If not assayed immediately, store lysed samples by freezing in glass vials with PTFE-faced septa, for example, 1 mL of lysed and filtered sample held in a 4mL vial.

## **7. INSTRUMENTATION AND EQUIPMENT**

- 7.1** Adda ELISA Test Kits- 96-well Microtiter Plates
  - 7.1.1** Microcystins/Nodularins- Abraxis PN 520011
  - 7.1.2** Microcystins-ADDA SAES- Abraxs PN 520011SAES
  - 7.1.3** Cylindrospermopsin- Abraxis PN 522011
  - 7.1.4** Standards



1. Microcystins ADDA: (6): 0, 0.15, 0.40, 1.0, 2.0, 5.0 ppb, 1mL each
  2. Microcystins ADDA SAES: (6): 0, 0.05, 0.15, 0.4, 1.5, 5.0 ppb, 1mL each
  3. Cylindrospermopsin: (7): 0, 0.05, 0.10, 0.25, 0.50, 1.0, 2.0 ppb, 1mL each
- 7.1.5 Control:**
1. Microcystins:  $0.75 \pm 0.185$  ppb, 1 mL
  2. Cylindrospermopsin:  $0.75 \pm 0.15$ ppb, 1 mL
- 7.1.6 Sample Diluent, 25 mL, for use as a Laboratory Reagent Blank and for dilution of samples above the range of the standard curve**
- 7.1.7 Antibody Solution**
1. Microcystins ADDA: 6mL
  2. Microcystins ADDA SAES, 6mL
  3. Cylindrospermopsin: rabbit anti-Cylindrospermopsin, 6 mL
- 7.1.8 Conjugate Solution**
1. Microcystins ADDA: Anti-Sheep-HRP conjugate solution, 12 mL
  2. Microcystins-ADDA SAES Conjugate Solution, 12mL
  3. Cylindrospermopsin: Cylindrospermopsin-HRP conjugate solution (vortex before use), 6 mL
- 7.1.9 Wash Buffer (5X) Concentrate, 100 mL, must be diluted prior to use**
- 7.1.10 Substrate (Color) Solution (TMB), 12 mL**
- 7.1.11 Stop Solution**
1. 6 mL for Microcystins
  2. 12mL for Cylindrospermopsin
- 7.1.12 Cylindrospermopsin Seawater Sample Treatment Solution, 45 test**
- 7.2 QuikLyse Cell Lysis for Microcystins/Nodularins ELISA microtiter plate**
- 7.2.1** Lysis Reagent A, 2.5 mL
  - 7.2.2** Lysis Reagent B, 0.5 mL
  - 7.2.3** Disposable Pipettes, 45
  - 7.2.4** Filtering Tips, 45
- 7.3 Cyanotoxin Manual Assay System- Abraxis PN 475010S. Includes:**
- 7.3.1** Microplate Reader, Model 4303
  - 7.3.2** Pipette, transfer, 10-100  $\mu$ L, adjustable
  - 7.3.3** Pipette, repeating, manual
  - 7.3.4** Pipette, multichannel, 8-tip, adjustable
  - 7.3.5** Basin, reagent, for multichannel, 50/bag
  - 7.3.6** Rack for 4mL vials, 48-postion (4x12)
- 7.4 Disposable plastic tips for pipettes**
- 7.4.1** Cartridges, Repeater, 1mL, bx/100- PN 70468
  - 7.4.2** Tips, Pipette, 10-200 $\mu$ L, 96/bx- PN 300002
  - 7.4.3** Tips, Pipette, 30-300 $\mu$ L, 96/bx- PN 300004
- 7.5 Vials for freezing samples**

- 7.5.1 Vials, Glass, Clear, 4 mL with caps
- 7.5.2 Vials, Glass, Clear, 40mL with caps
- 7.6 Syringes and Filters for Lysing
  - 7.6.1 All plastic Luer-Lok syringes, 3mL, from Thermofisher Scientific
  - 7.6.2 Glass Fiber Syringe Filters, 25mm, 1.2 $\mu$ m,
- 7.7 500 mL PETG containers with PTFE septa lined lids
- 7.8 Parafilm for plate covering

## 8. REAGENTS, STANDARDS, AND CONSUMABLE MATERIALS

### 8.1 Analysis Kit

Store kits according to manufacturer's instructions. Standards and reagents may be used until the manufacturer's expiration date.

- 8.1.1 Both the Microcystin and Cyindrospermopsin kits should be stored in the refrigerator (4-8°C). The solutions must be allowed to reach room temperature (20-25 °C) before use. Consult state, local, and federal regulations for proper disposal of all reagents.
- 8.1.2 QuikLyse reagents should be stored in the refrigerator (2-8°C). The remaining components in the QuikLyse kit require no special storage conditions and may be stored separately from the reagents to conserve refrigerator space. Discard samples according to local, state, and federal regulations. Allow the QuikLyse reagents to warm to room temperature before use.

## 9. INSTRUMENT CALIBRATION PROCEDURES

### 9.1 Micropipettors

Micropipettors must be verified each year for accuracy. Verification of accuracy is done by pipetting DI water and then weighing to determine if it is accurate. This check must be done for 50 $\mu$ L, 100  $\mu$ L, and 250  $\mu$ L.

### 9.2 Calibration Procedure

A calibration is required with each Analysis Batch. Use the concentrations stated in the kit instructions. Do not add additional calibration levels or eliminate any levels. Use the calibration standards provided in the original kit. Each calibration standard must be added to at least two wells.

### 9.3 Calibration Acceptance Criteria

The calibration curve is validated by evaluating the %CV of the absorbance values for the well replicates representing each calibration level, and the correlation coefficient of the four-parameter logistic curve. Calculate the %CV for each of the paired absorbance values, including the "zero" standard. The %CV for each pair must be less than, or equal to, 10%. However, one pair is allowed to exceed 10% providing the %CV is less than, or equal to, 15%. The square of the correlation coefficient ( $r^2$ ) of the four-parameter curve must be greater than, or equal to, 0.98.

If the calibration fails, the %CV limits or  $r^2$  is less than 0.98, then the entire Analysis Batch is invalid. Assay the samples in a subsequent Analysis Batch.

Freeze the filtered samples if this Analysis Batch cannot be completed on the same day as the original attempt. Each sample must be within the 14-day holding time for the repeat assay.

## **10. Procedures**

### **10.1 Sample Lysing Procedure by Freeze-Thaw**

**10.1.1** Mix samples thoroughly and immediately transfer 5 to 10 mL of each field sample into a 40 mL vial to begin three freeze-thaw cycles. If the sample was previously frozen, only two freeze-thaw cycles are needed (once it has thawed, it has undergone the first freeze/thaw cycle). Smaller vials may be use but reduce the sample volume to less than 25% of vial capacity.

**10.1.2** Once sample is completely frozen, remove from freezer and thaw. To speed up the process, vials may be immersed in a 35°C in a water bath until completely thawed. Ensure samples are completely frozen and completely thawed during each cycle.

**10.1.3** Filter 1 to 2 mL of each lysed sample into a 4mL vial using a glass-fiber syringe filter. Samples are ready for immediate analysis.

### **10.2 Sample Lysing Procedure by Abraxis QuikLyse**

**10.2.1** Transfer 1 mL of sample to a glass vial

**10.2.2** Add 100 uL of QuikLyse Reagent A to the sample in the vial. Cap and shake for 2 minutes. Incubate for 8 minutes at room temperature.

**10.2.3** Add 10 uL of QuikLyse Reagent B to the sample in the vial. Cap and shake for 2 minutes. Incubate for 8 minutes at room temperature.

**10.2.4** Draw less than half of the treated sample into a disposable pipette. Place a filtering tip firmly onto the disposable pipette. Sample will leak if pipette and tip are not pressed tightly together.

**10.2.5** Squeeze the pipette bulb gently, filtering the sample dropwise into a clean glass vial. The filtering tip can be removed and reattached to filter the entire lysed sample, if desired

**10.2.6** The lysed, filtered sample is now ready for analysis with one of the Abraxis Microcystins ELISA Microtiter Plate Kits.

- 1.** Results obtained with samples prepared using the QuikLyse system must be multiplied by 1.11 to correct for sample dilution from the QuikLyse reagents.

### **10.3 Seawater Sample Preparation**

#### **10.3.1 Microcystins**

- 1.** No matrix effects have been observed with seawater salinities (salinity up to 38 parts per thousand) using the ADDA SAES ELISA plate

#### **10.3.2 Cylindrospermopsin**

1. Weigh 0.1 g of Cylindrospermopsin Seawater Sample Treatment reagent into a clean, appropriately labeled 4mL glass vial
2. Add 1mL of brackish water or seawater sample to the vial
3. Vortex for 1 minute. Allow the sample to settle for 10 minutes
4. Pipette the supernatant into an appropriately labeled microcentrifuge tube. Centrifuge for 5 minutes at 13,000 rpm. The sample will separate into 3 layers: a solid, white precipitate (bottom layer), a clear liquid (center layer), and a very thin white film (on top of the liquid layer).
5. Pipette the clear liquid (center layer) into a clean, appropriately labeled 4mL glass vial. Avoid pipetting the very thin white film
6. Dilute the supernatant 1: 3 with DI H<sub>2</sub>O (I.e. 333 uL supernatant and 667 ul DI H<sub>2</sub>O). The sample can then be analyzed using the Abraxis Cylindrospermopsin ELISA Kit.

#### **10.4 Test Preparation**

- 10.4.1 Verify kit standards and reagents are used prior to the expiration date. Allow the reagents and samples to reach ambient temperature before analysis. The assay procedure must be performed away from direct sunlight.
- 10.4.2 Remove the number of microtiter plate strips required from the resealable pouch. The remaining strips are stored in the pouch with the desiccant (tightly sealed)
- 10.4.3 The standards, control, sample diluent, antibody enzyme conjugate, substrate, and stop solutions are ready to use and do not require any further dilutions
- 10.4.4 Dilute the wash buffer (5X) concentrate at a ratio of 1:5 with deionized or distilled water. If using the entire bottle (100mL), add to 400mL of deionized or distilled water and mix thoroughly.
- 10.4.5 The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously. See Table 1.

#### **10.5 Assay Procedures**

##### **10.5.1 Microcystins**

1. Add 50µL of the standard solutions, control, or samples into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add 50µL of the antibody solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the

contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 90 minutes at room temperature.

3. Remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. Wash the strips three times using the diluted wash buffer. Please use at least a volume of 250  $\mu\text{L}$  of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
4. Add 100  $\mu\text{L}$  of the enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strip for 30 minutes at room temperature.
5. Remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. Wash the strip three times using the diluted wash buffer. Please use at least a volume of 250  $\mu\text{L}$  of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
6. Add 100  $\mu\text{L}$  of substrate (color) solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 20-30 minutes at room temperature. Protect the strips from sunlight.
7. Add 50  $\mu\text{L}$  of stop solution to the wells in the same sequence as for the substrate (color) solution using a multi-channel pipette or a stepping pipette.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

#### **10.5.2 Cylindrospermopsin**

1. Add 50  $\mu\text{L}$  of the standards, control (QCS), LRB, or samples into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.

2. Add 50  $\mu\text{L}$  of the enzyme conjugate solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette.
3. Add 50  $\mu\text{L}$  of the antibody solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 45 minutes at room temperature.
4. Remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. Wash the strips four times using the diluted wash buffer. Please use at least a volume of 250  $\mu\text{L}$  of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
5. Add 100  $\mu\text{L}$  of substrate (color) solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells in the same sequence as for the substrate (color) solution using a multi-channel, stepping or electronic repeating pipette.
6. Add 100  $\mu\text{L}$  of stop solution to the wells in the same sequence as for the substrate (color) solution using a multi-channel, stepping, or electronic repeating pipette.
7. Read the absorbance at 450nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

## **10.6 Running an Assay**

- 10.6.1** Place the plate instrument with well A-1 at the rear right corner so that row 1 is going into the reader first. As you press the first row back and down you will feel slight tension on the plate stretching the carrier so that the front fits in. The plate requires a snug fit.
- 10.6.2** When using a strip tray, make sure wells are pushed down into tray so that they will not cause the plate to jam or entry. Use care that well tabs do not extend over other wells. Do not place the tabbed ends of strips in row 1; they should be in row 12. Be sure to place the strips in the order in which Blanks, Calibrators and Samples are to be read.
- 10.6.3** For best results, do not fill wells completely; 200-250  $\mu\text{L}$  depending on well total volume is the maximum fill recommended when the mixing feature is used.
- 10.6.4** Plate Layout is the default window for Abraxis Reader and displays when the program is started. There are several options: Load Plate, Save Plate, Reset, Re-Assign, Read Plate or Remove. Once samples

have been assigned, press the Read Plate button to run. Results are displayed as delta Abs for fixed time read, and delta Abs/min for non-fixed time kinetic. Refer to the “AReader Abraxis Model 4303 Operators Manual” for more information on running an assay.

**10.6.5** Sample analyses resulting in a higher concentration than the highest standard in the calibration curve must be diluted within the calibration range and reanalyzed to obtain accurate results. Samples may not be diluted in the well plate. If a sample is diluted, the final values must be calculated by multiplying the result by the proper dilution factor. Report calculated values.

**10.6.6** Save and print a copy of the calibration curve and sample results as part of the laboratory’s record maintenance protocol.

**10.6.7** Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the standards.

**10.4.7.1** Samples with lower absorbances than a standard will have concentrations of Microcystins or Cylindrospermopsin greater than the standard. Samples which have higher absorbances than a standard will have concentrations of Microcystins or Cylindrospermopsin less than that standard.

## **10. 5 QUALITY CONTROL**

QC requirements include the IDC, and QC elements associated with each Analysis Batch. This section describes each QC parameter, its required frequency, and the performance criteria that must be met in order to satisfy EPA data quality objectives. These QC requirements are considered the minimum acceptable QC protocol. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

### **10.5.1 Initial Demonstration of Capability (IDC)**

The IDC must be successfully performed prior to analyzing field samples. A plate with all calibration standards, controls, and LRB, plus 10 field samples, must be ran in duplicate wells for the IDC. The IDC must be performed by each analyst, when a new analyst begins work or whenever a change in analytical performance.

When conducting the IDC, the analyst must meet the calibration requirements specified in section 9 for the standards. The %CV for each pair must be less than, or equal to, 10%. However, one pair is allowed to exceed 10% providing the %CV is less than, or equal to, 15%. All samples must have a %CV of less than 15%. If the analyst fails to meet the %CV limits or  $r^2 = 0.98$  for the given standards, then their batch is invalid and they must perform the analysis in a subsequent Analysis Batch. The mean recovery of the QCS must also have a percent recovery  $\geq 70\%$  and  $\leq 130\%$  of the true value. If the analyst fails to meet the percent recovery during the IDC, then the analysis batch is invalid and must be performed again in a subsequent Analysis Batch.

### 10.5.2. Criterion for Replicate Wells

All field and QC samples are added to at least two wells. The %CV of the absorbance values measured for the well replicates must be less than, or equal to, 15%. Calculate the %CV as follows:

$$\%CV = \frac{\text{Standard Deviation of Absorbances}}{\text{Mean Absorbance}} \times 100\%$$

If the %CV exceeds 15% for a field sample or QC sample, then that sample is invalid. Note that the well replicates of calibration standards must meet a different set of criteria for %CV.

### 10.5.3 Quality Control Standard (QCS)

A secondary source QCS must be analyzed with each batch of samples to verify the concentration of the calibration curve. If a QCS is already included in the kit, it may be used if it has a different lot number than the calibration standards and was prepared from a separate primary stock. Acceptance limits must be within  $\pm 25\%$  of true value. QCS values exceeding the acceptance limits require action and reanalysis of sample(s) with results greater than the concentration of an acceptable Low-CV in the same analytical batch. If reanalysis is not possible, all sample concentration results greater than an acceptable Low-CV analyzed in the same batch must be appropriately qualified and noted in the final report.

## 11 DATA REDUCTION, VALIDATION, AND REPORTING

### 11.1 Quantitation

A four-parameter logistic curve fit must be used. Other curve-fitting models are not permitted. Calculate the sample concentration for each well using the multipoint calibration. For each field and QC sample, average the two concentration values from each well. Use this mean to report sample results and to evaluate QC results against acceptance limits. Final results should be rounded to two significant figures.

### 11.2 Exceeding the Calibration Range

If a result exceeds the range of the calibration curve, dilute the sample with reagent water. Analyze the diluted sample in a subsequent Analysis Batch. Incorporate the dilution factor into the final concentration calculations. Report the dilution factor with the sample result.

## 12 WASTE MANAGEMENT

The EPA requires that laboratory waste management practices be consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. In addition, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions.



**13 REFERENCES**

EPA Method 546, “*Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay*”; EPA 815-B-16-011; Office of Water: Cincinnati, OH, August 2016.

**14 REVISION HISTORY**

<b>Revision</b>	<b>Date</b>	<b>Summary</b>	<b>Section</b>
1	03/05/20	Added limit detection for Microcystins ADDA-SAES and for use of Cylindrospermopsin seawater sample treatment	1.1
1	03/05/2020	Added safety information about the Cylindrospermopsin seawater sample treatment	4.3
1	03/05/20	Added limitations with methanol	5.2
1	03/05/20	Changed 1 L PETG container to 500mL	6.1
1	03/05/20	Added Microcystins ADDA-SAES test kit supplies	7.1
1	03/05/20	Added Cylindrospermopsin seawater sample treatment to supplies	7.1.12
1	03/05/20	Changed 1 L PETG container to 500mL	7.6
2	02/28/22	Add QuikLyse to list of equipment	7.2
2	02/28/22	Add QuikLyse reagents	8.1.2
2	02/28/22	Add QuikLyse procedure	10.2

**15 Tables, Figures, and Method Performance Data****Table 1. Working Scheme of microtiter plate**

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	<b>Std 0</b>	<b>Std 4</b>	<b>Sample 2</b>									
<b>B</b>	<b>Std 0</b>	<b>Std 4</b>	<b>Sample 2</b>									
<b>C</b>	<b>Std 1</b>	<b>Std 5</b>	<b>Sample 3</b>									
<b>D</b>	<b>Std 1</b>	<b>Std 5</b>	<b>Sample 3</b>									
<b>E</b>	<b>Std 2</b>	<b>Control</b>	<b>Etc.</b>									
<b>F</b>	<b>Std 2</b>	<b>Control</b>	<b>Etc.</b>									
<b>G</b>	<b>Std 3</b>	<b>Sample 1</b>										
<b>H</b>	<b>Std 3</b>	<b>Sample 1</b>										

\*\* Note: The working scheme of the Cylindrospermopsin plate contains an additional standard. Thus well G2 and H2 will be used for Standard 6 and the samples will start in the wells in column 3.

**Table 2. Analysis Batch QC Requirements**

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
9	ELISA Calibration- with provided standards	Use kit-recommended levels and concentrations. Two well replicates per standard.	%CV of absorbance $\leq 10\%$ ; $\leq 15\%$ allowed for 1 pair. $r^2 \geq 0.98$
3.2	Well Replicates	Assay field and QC samples in two wells	Sample invalid if %CV of absorbance values $> 15\%$
3.11	Quality Control Sample (QCS)	Assay 1 QCS for each new lot of calibration standards. Prepare the QCS near the $EC_{50}$ with MC-LR from a source independent of the calibration standards.	Percent recovery $\geq 70\%$ and $\leq 130\%$ of the true value.

**Appendix 2:** Results of 2021 microcystin analyses, which are organized by water body, sites within those water bodies, and the analytical results for each of the sites based on the sampling month.

Water Body	Site	Microcystin Concentration (µg/L) <sup>a</sup>						
		Apr	May	Jun	Jul	Aug	Sep	Oct
Ashepoo River	MD-253	- <sup>b</sup>	BDL	-	0.0475	0.0275	-	-
Ashley River	MD-049	-	0.0825	0.095	0.066	0.0635	0.0265	-
	CSTL-102	-	0.1185	0.0835	0.1055	0.082	-	-
	MD-052	-	BDL	0.046	0.068	0.0655	BDL	-
Beaufort River	MD-001	-	0.0465	-	0.0325	0.0485	0.02	BDL
	MD-004	-	0.0615	-	0.0315	0.062	0.0325	0.036
Black River	PD-325	-	BDL	0.0565	0.064	0.091	0.038	0.016
Bohicket Creek	MD-209	-	0.017	BDL	0.074	0.0215	BDL	-
Boyd Mill Pond	S-311	-	0.0955	0.019	0.0525	0.031	0.0245	BDL
Broad Creek	MD-174	-	0.02	BDL	0.068	BDL	-	-
Broad River	MD-116	-	0.018	-	0.071	-	BDL	0.0175
Casino Creek	MD-266	-	0.0355	0.031	0.062	BDL	0.043	0.0535
Cedar Creek Reservoir	CW-033	-	0.075	0.0755	0.174	0.1255	BDL	0.099
	CW-174	-	0.0845	0.1255	0.1705	0.1375	BDL	0.1235
Chechessee	MD-117	-	BDL	-	0.1735	BDL	BDL	0.0245
Colleton River	MD-176	-	BDL	-	0.0785	BDL	BDL	0.018
Combahee River	MD-252	-	0.0275	-	0.0415	0.037	-	-
Cooper River	MD-043	-	BDL	0.085	0.108	0.1875	0.0615	-
	MD-045	-	0.024	0.0425	0.05	0.0505	0.025	-
	MD-248	-	BDL	0.043	0.074	0.1495	0.0495	-
Coosawhatchie River	CSTL-107	-	0.0315	-	0.092	0.1235	0.035	0.049
Dawho River	MD-120	-	0.039	BDL	0.073	0.0185	BDL	-
Fishing Creek Reservoir	LCR-04	-	0.085	0.092	0.1495	BDL	BDL	BDL
	CW-016F	-	0.056	-	0.2055	0.148	0.136	BDL
	CW-057	-	0.0555	0.1075	0.174	0.1135	BDL	0.141
Five Fathom Creek	MD-267	-	0.044	BDL	0.0505	BDL	0.1225	0.1995
Folly River	MD-130	-	BDL	BDL	0.039	BDL	BDL	-
Great Swamp	MD-129	-	0.0255	-	0.0575	0.0545	BDL	0.034
Hamlin Sound	MD-271	-	0.021	0.028	0.028	BDL	0.0435	0.0335
Intracoastal Waterway	MD-069	-	BDL	BDL	0.0235	BDL	0.0285	BDL
	MD-125	-	0.0185	0.057	0.0735	0.0465	BDL	0.0205
J. Strom Thurmond	CL-041	-	0.135	0.1925	0.283	0.253	0.178	0.143
Kiawah River	MD-273	-	0.0435	BDL	0.068	BDL	BDL	-

Water Body	Site	Microcystin Concentration (µg/L) <sup>a</sup>						
		Apr	May	Jun	Jul	Aug	Sep	Oct
Lake Bowen	B-339	-	0.018	0.066	0.1045	0.128	0.1065	-
Lake Greenwood	S-022	-	0.0355	0.0965	0.081	0.086	BDL	0.074
	S-024	-	BDL	0.0305	0.0685	0.1285	0.1165	0.0475
	S-131	-	0.0445	0.0485	0.028	0.127	0.1155	0.082
	S-308	-	0.037	0.062	0.1005	0.1445	0.051	0.0615
Lake Hartwell	SV-200	-	0.049	0.0485	0.053	BDL	BDL	BDL
	SV-236	-	0.0655	0.0995	0.113	BDL	0.0305	-
	SV-268	-	0.064	0.016	0.035	0.0605	BDL	BDL
	SV-339	-	0.0465	0.0535	0.1315	0.0895	0.0505	0.124
	SV-340	-	0.035	0.032	0.0605	0.067	0.069	0.1385
	SV-363	-	0.11	0.092	0.0795	BDL	0.0865	0.1035
	SV-374	-	0.04	0.085	0.218	0.129	0.0455	0.0835
Lake Jocassee	CL-019	-	0.0165	BDL	0.0335	BDL	BDL	BDL
	SV-335	-	0.022	BDL	0.024	BDL	BDL	0.02
	SV-336	-	0.0205	0.024	BDL	BDL	0.0395	0.01655
Lake Keowee	SV-338	-	BDL	0.073	0.0415	BDL	0.0645	-
	SV-361	-	0.032	0.0355	0.058	0.1235	0.043	BDL
Lake Murray	S-211	-	0.1465	0.1615	0.2705	0.1515	0.1005	0.2595
	S-213	-	0.158	0.141	0.3355	0.150	0.165	0.246
	S-222	0.367	-	0.183	0.221	0.1495	0.0995	0.1075
	S-223	-	-	0.047	0.1745	0.125	0.048	BDL
	S-279	0.0635	0.172	0.1725	0.352	0.134	0.128	0.189
	S-280	-	0.148	0.2285	0.347	0.182	0.147	0.229
	S-309	0.0535	0.108	0.1405	0.2545	0.2085	0.085	0.1075
	S-310	-	-	0.0235	0.1145	0.1055	0.039	BDL
	S-326	0.5	0.2245	0.233	0.285	0.159	0.166	0.1025
RL-19154	0.323	0.321	0.188	0.274	0.1205	0.183	BDL	
Lake Robinson	PD-327	-	0.1	0.0595	0.049	0.067	0.067	BDL
Lake Russell	SV-098	-	0.061	0.1085	0.173	0.2455	0.055	0.1135
	SV-357	-	0.0885	0.2305	0.286	0.259	0.057	0.0565
Lake Secession	SV-331	-	0.0235	0.174	0.1155	0.064	0.0725	BDL
Lake Wateree	CL-089	-	0.245	0.1325	-	0.1575	0.1035	0.2795
	CW-207	-	0.151	0.139	-	0.177	0.144	0.2715
	CW-207B	-	0.159	0.1455	-	0.198	0.191	0.356
	CW-208	-	0.125	0.095	-	0.1835	0.096	0.2455
	CW-231	-	0.0685	0.117	0.136	0.1205	-	0.1815
	LCR-02	-	0.0865	0.069	-	0.182	0.0355	0.125
	LCR-03	-	0.1105	BDL	-	0.149	0.106	0.1025
Lake Wylie	CW-197	-	BDL	0.075	0.2115	0.1375	0.0935	0.16
	CW-201	-	0.168	0.187	0.343	0.127	0.1375	0.155
	CW-230	-	0.117	0.1595	0.373	BDL	0.1435	0.1525

Water Body	Site	Microcystin Concentration ( $\mu\text{g/L}$ ) <sup>a</sup>						
		Apr	May	Jun	Jul	Aug	Sep	Oct
Langley Pond	CL-069	-	0.0645	0.068	0.07	0.1875	BDL	BDL
May River	MD-173	-	BDL	BDL	0.019	BDL	-	-
Monticello Lake	B-327	-	0.115	0.1325	0.135	BDL	0.236	BDL
Morgan River	MD-282	-	0.0185	BDL	0.0575	BDL	0.0475	0.016
N. Edisto River	MD-262	-	0.04	0.0175	0.054	-	BDL	-
New River	MD-118	-	0.0275	-	0.0345	0.056	0.024	0.0985
Parr Reservoir	B-345	-	0.0605	0.0685	0.064	BDL	0.0435	BDL
Parrot Creek	MD-281	-	0.0285	BDL	0.0415	BDL	0.0225	0.1225
Parsonnage Creek	MD-277	-	BDL	0.053	0.0895	BDL	0.0255	BDL
Pee Dee River	MD-275	-	0.021	0.061	0.0825	0.0345	0.0645	0.0335
Ramshorn Creek	MD-257	-	0.0225	BDL	BDL	BDL	BDL	-
	MD-258	-	0.026	0.022	BDL	BDL	0.0225	-
Santee River	ST-005	-	0.031	0.116	-	0.0865	0.1005	0.12
	ST-006	-	-	0.076	-	0.049	0.075	0.1225
S. Edisto River	MD-260	-	BDL	BDL	0.078	BDL	BDL	-
Sampit River	MD-077	-	BDL	0.0275	0.0845	0.041	0.0215	BDL
Sewee Bay	MD-269	-	BDL	BDL	0.0175	BDL	0.0625	0.0385
Stephens Creek Reservoir	SV-372	-	0.054	0.084	0.0855	0.127	0.094	0.114
Stono River	MD-202	-	0.036	0.077	0.025	BDL	0.022	-
	MD-206	-	0.043	BDL	0.044	BDL	BDL	-
Unnamed Creek	MD-256	-	0.0295	-	0.016	BDL	-	-
Waccamaw River	MD-142	-	0.0165	0.0635	0.08	0.054	0.03	0.0215
Wando River	MD-115	-	BDL	0.021	0.0485	BDL	0.0165	-
	MD-264	-	0.026	0.0525	0.0295	0.072	0.022	-
Winyah Bay	MD-278	-	BDL	BDL	BDL	BDL	0.038	BDL
Wright River	MD-259	-	0.027	0.0275	0.0275	0.0165	0.0225	-
Yonges Island Creek	MD-261	-	BDL	0.0275	0.053	0.0385	BDL	-

a.  $\mu\text{g/L}$  = micrograms per liter (parts per billion)

b. Dashes indicate no data available

c. BDL= below detection limit

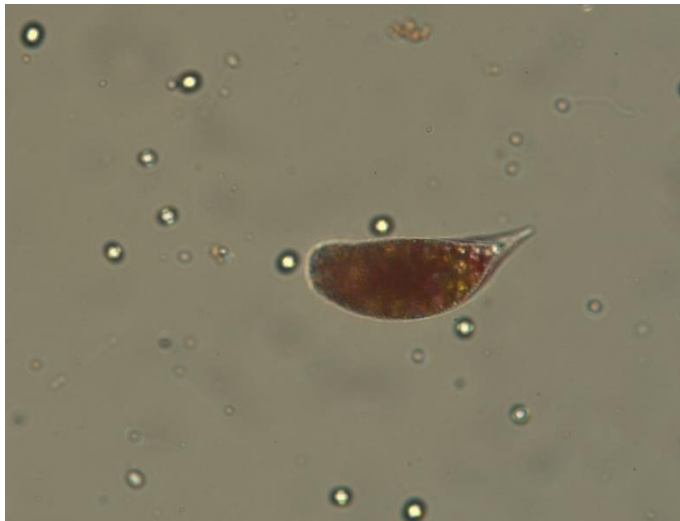
**Appendix 3:** Microscopic images of cyanobacteria from the 2021 HAB complaint sites.



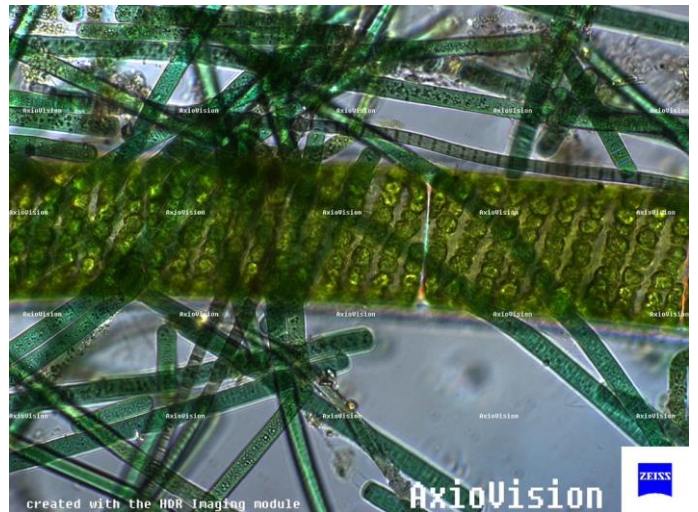
*Aphanizomenon* sp. bloom at  
Goose Creek Reservoir on 04/08/2021.



*Dolichospermum* sp. bloom at  
Goose Creek Reservoir on 11/17/2021.



*Euglena sanguinea* bloom at  
Camp Canaan on 05/13/2021.



*Phormidium* sp. bloom at  
Lake Wateree 07/09/2021.

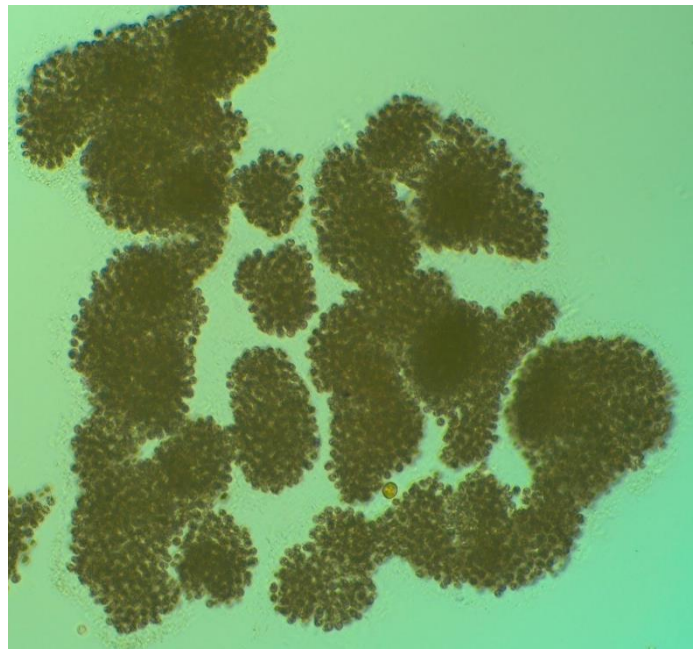
**Appendix 4:** Recreational Watches issued on Goose Creek Reservoir and Lake Wylie. Samples were collected monthly at the waterbody until the bloom was no longer present.

Lake Name	Location	HAB description	Associated algal toxins	Watch Issued	Watch Lifted
Goose Creek Reservoir	Entire lake	<i>Aphanizomenon sp.</i>	Cylindrospermopsin, Anatoxin, Saxitoxin	04/08/2021	06/01/2021
Lake Wylie	9 coves on the lake	<i>Microcystis sp.</i>	Microcystins	10/08/2021	11/16/2021
Goose Creek Reservoir	Entire lake	<i>Dolichospermum sp</i>	Cylindrospermopsin, Microcystins, Anatoxin, Saxitoxin	11/15/2021	12/20/2021

**Appendix 5: Images from the HAB on Lake Wylie.**



Algal bloom in a Lake Wylie Cove.



Microscope image of the Lake Wylie HAB (*Microcystis* sp.).



Drone Image of an algal bloom in a cove on Lake Wylie.